PB82-109356

Study of Chlorine Dioxide and Its Metabolites in Man

Ohio State Univ., Columbus

Prepared for

Health Effects Research Lab.
Cincinnati, OH

Sep 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

PB82-109356

EPA-600/1-81-068

STUDY OF CHLORINE DIOXIDE AND ITS METABOLITES IN MAN

by

Joseph R. Bianchine, Judith R. Lubbers,
Sudha Chauhan and Judy Miller
Department of Pharmacology
Ohio State University
College of Medicine
Columbus, Ohio 43210

Grant Number 805643

Project Officer

Richard Bull, Ph.D.
Toxicological Assessment Branch
Laboratory Studies Division
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

NOTICE

THIS DOCUMENT HAS BEEN REPRODUCED FROM THE BEST COPY FURNISHED US BY THE SPONSORING AGENCY. ALTHOUGH IT IS RECOGNIZED THAT CERTAIN PORTIONS ARE ILLEGIBLE, IT IS BEING RELEASED IN THE INTEREST OF MAKING AVAILABLE AS MUCH INFORMATION AS POSSIBLE.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-81-068	ORD Report	PB82 109356

4. TITLE AND SUBTITLE	5. REPORT DATE
Study of Chlorine Dioxide and its Metabolites in Man	September 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
J.R.Bianchine et al.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Department of Pharmacology Ohio State University College of Medicine Columbus, Ohio 43210	11. CONTRACT/GRANT NO. 805643
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. EPA Health Effects Research Laboratory 26 W. St. Clair St. Cincinnati, Ohio 45268	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT To assist in the assessment of the relative safety of chronically administered chlorine water disinfectants in man, a controlled study was undertaken. The study was conducted in three phases. Phase I, a rising dose tolerance investigation, examined the effects of single dose increasing concentration administration of chlorine disinfectants to normal healthy adult male volunteers. Phase II considered the impact on normal subjects of twelve week daily ingestion of the disinfectants at a concentration of 5 mg/L. In phase III, chlorite, at a concentration of 5 mg/L, was administered daily to glucose-6-phosphate dehydrogenase deficient subjects. Physiological impact was assessed by evaluation of a large battery of qualitative and quantitative tests.

In general, the study affirmed the relative safety and tolerance of normal healthy adult males and normal healthy adult male G-6-PD deficient individuals to daily twelve week ingestion of 500 mL of chlorine disinfectants at a concentration of 5 mg/L.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	101
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

DISCLAIMER

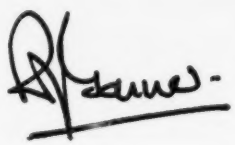
This report has been reviewed by the Laboratory Studies Division, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise, and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The Laboratory Studies Division contributes to this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

These carefully controlled and maintained studies are designed to evaluate the safety of chlorine dioxide (a potential water disinfectant) and its metabolites in normal human volunteer subjects.



R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

Chlorine dioxide (ClO_2) has been identified as a potential water disinfectant alternative to chlorination. A three-phase human investigation was undertaken to assess the safety of ClO_2 and its metabolites.

In Phase I: Rising-Dose Tolerance Study, a controlled double-blind study was initiated to assess the safety of single dose administration of chlorine dioxide and its metabolites over a broad range of concentrations. Normal healthy adult males were treated with successive increasing doses of chlorine dioxide, chlorite, chlorate, chloramine, chlorine or untreated water (control). The concentration ranges examined were as follows: chlorine dioxide, 0.1 to 24.0 ppm; chlorite, 0.01 to 12.0 ppm; chlorate, 0.01 to 12.0 ppm; chloramine, 0.01 to 24.0 ppm and chlorine, 0.01 to 24.0 ppm.

In Phase II: Chronic Administration to Normal Subjects. Sixty volunteers were divided, at random, into six double-blind treatment groups of 10 subjects each for the administration of ClO_2; ClO_2 metabolites, chlorite (ClO_2^-) and chlorate (ClO_3^-); chloramine (NH_2Cl); chlorine; and control, distilled water. The 20-week protocol involved a 12-week period of daily oral administration of 500 mL of treated water followed by an 8-week observation period. The concentration of water disinfectants was 5 mg/L. Urine and blood samples collected at weekly intervals were subjected to complete routine clinical analysis and to special determinations for methemoglobin, glutathione, glucose-6-phosphate dehydrogenase and thyroid function. Subjective evaluation of the qualitative clinical tests was coupled with the statistical evaluation of all quantitative chemical parameters.

In Phase III: Chronic Administration to G-6-PD Deficient Subjects. Three healthy male volunteers found to be deficient in glucose-6-phosphate dehydrogenase were chosen for the study. All three subjects ingested chlorite; no concurrent control study was performed. The study protocol was identical to that of Phase II in temporal design and evaluation procedures. Volunteers received 500 mL daily of sodium chlorite at a concentration of 5 mg/L chlorite.

In each of the three phases of the study, numerical analyses revealed statistically significant trends. Before any of these changes with respect to time may be attributed with confidence to a physiological response to ingestion of chlorine disinfectants, one must take into consideration certain pertinent facts:

1) In most cases, the precision of laboratory measurements of biochemical parameters exceeded the range of physiological

bioregulation. A statistically significant trend, therefore, may or may not have physiological importance.

2) The chronic studies included only twelve weeks of treatment. In actual use of the disinfectants in water supplies over a longer period of time, the statistically significant trends could possibly achieve proportions of biological consequence.

Therefore, it is not possible to attribute the trends with confidence to the impact of oral disinfectant ingestion, nor is it reasonable to consider them of biological significance. However, one cannot rule out the possibility that, over an extended period of time, the trends could continue and adversely affect the health of the recipients.

In general, the study affirmed the relative safety and tolerance of normal healthy adult males and normal healthy adult male G-6-PD deficient individuals to daily twelve week ingestion of 500 mL of chlorine disinfectants at a concentration of 5 mg/L.

CONTENTS

Foreword iii
Abstract ..... iv-v
Contents vii
Tables viii
Figures ix
Acknowledgments x

Introduction 1
Conclusion 2
Recommendation 3
Contents 4
Objective 4
Materials and Methods 4
Subjects selection 4
Concurrent drug therapy 5
Institutional review committee 5
Informed consent form 5
Disinfectant preparation 5
Stock solutions 6
ClO_2 6
Cl_2 6
NH_2Cl 6
ClO_2^- and ClO_3^- 6
Chemicals 6
Study design 7
Phase I 7
Phase II 7
Phase III 8
Evaluation 8
Statistical analysis 8

Results 8
Qualitative 8
Quantitative 9
Vital signs 9
Biochemical parameters 9
Phase I 9
Phase II 12
Phase III 14
Palatability 14

Discussion 15

Bibliography 16

TABLES

Number		Page
1	Concentration of Disinfectants and Subject Assignment (Phase I)	18
2	Concentration of Disinfectants and Subject Assignment (Phase II)	19
3	Schedule for Evaluation Procedures (Phase I)	20
4	Schedule for Evaluation Procedures (Phases II and III)	21
5	Battery of Laboratory Tests and Normal Ranges	22
6	Two-Way Analysis of Variance with Repeated Measures; Comparison of Treatment Groups to the Control Group (Phase I)	26
7	Selected Group Mean Values and Standard Deviations from the Mean (Phase I)	28
8	Omnibus Test (Phase II)	30
9	Analysis of Variance with Repeated Measures (Phase II)	34
10	Linear Regression Analysis (Phase II)	36
11	Linear Regression Analysis (Phase III)	37
12	Palatability (Phase I)	38

FIGURES

Number		Page
1	Mean Corpuscular Hemoglobin: chlorate vs control (Phase II)	40
2	Mean Corpuscular Hemoglobin: chlorite vs control (Phase II)	41
3	Urea Nitrogen: chlorine dioxide vs control (Phase II)	42
4	Urea Nitrogen: chlorate vs control (Phase II)	43
	Supplementary Figure Appendix	44

ACKNOWLEDGMENTS

The cooperation of the entire clinical pharmacology unit of The Ohio State University, College of Mecicine was essential to the successful completion of this study. Many individuals contributed very important services to this difficult clinical study. We gratefully acknowledge the excellent assistance of the following:

David A. Brys, Mary T. Coleman, Jeffrey M. Dicke, M.D., John A. Difini, Nancy Dorman, Patrick B. Doyle, Mary A. Gravenstein, Stefan Gravenstein, Henry T. Grinvalsky, M.D., Stephen R. Guy, Terrence Harding, Robert H. Hunsaker, K. Jean Joviak, Douglas A. Kuperman, Paul C. Levy, George Lewandowski, Robert P. Lynch, Michael L. Markel, Katherine Oyakawa, Christine Pacula, Jose A. Ramirez, Daniel M. Slutzker, James Smith, Mary E. Smith, Michael Y. Zorko, Ann Zorko.

The computer-assisted statistical analysis of data was performed with the aid of Jerry Martin and Michael Oskins of the OSU, Division of Computing Services for Medical Education and Research under the supervision of Pentti Karki.

Dr. Richard Bull and his staff provided especially valuable guidance and suggestions throughout this entire study. We are deeply grateful for this continual interest and support.

Ms. Aline Marleau provided valuable editorial services while Carol Jones and Mary Ellen Wetherhold provided fine secretarial assistance throughout the study.

INTRODUCTION

To assist in the assessment of the relative safety of chronically administered chlorine water disinfectants in man, a controlled study was undertaken. The study was conducted in three phases. Phase I, a rising dose tolerance investigation, examined the effects of single dose increasing concentration administration of chlorine disinfectants to normal healthy adult male volunteers. Phase II considered the impact on normal subjects of twelve week daily ingestion of the disinfectants at a concentration of 5 mg/L. In phase III, chlorite, at a concentration of 5 mg/L, was administered daily to glucose-6-phosphate dehydrogenase deficient subjects. Physiological impact was assessed by evaluation of a large battery of qualitative and quantitative tests.

CONCLUSION

All three phases of this large controlled and double blinded clinical evaluations of chlorine dioxide and its potential metabolites in human male volunteer subjects were completed uneventfully. There were no obvious undesirable clinical sequellae noted by any of the participating subjects or the observing medical team. In several cases, statistically significant trends in certain biochemical or physiological parameters were associated with treatment; however, none of these trends were judged to have physiological consequence. One cannot rule out the possibility that, over a longer treatment period, these trends might indeed achieve proportions of clinical importance. However, within the limits of the study, the relative safety of oral ingestion of chlorine dioxide and its metabolites, chlorite and chlorate, was demonstrated.

RECOMMENDATIONS

This study was limited in some respects:

1) Only male volunteers participated.
2) Treatment was limited to twelve weeks.
3) The number of G-6-PD subjects was very small.
4) The concentration of disinfectants was fairly low.

With this in mind, we make several recommendations. In use of chlorine dioxide as a water disinfectant, the entire population will ingest the disinfectant and its byproducts over a long period of time. The safety of ingestion in women and children must be assessed. The length of the study must be increased. Furthermore, on the basis of Phase III, the absence of detrimental effects on potentially susceptible individuals cannot be ascertained with confidence; the sample size was limited and no concurrent control was run. If chlorine dioxide is to be used as a primary disinfectant, human investigations at increased dose levels ought to be conducted.

CONTENTS

OBJECTIVE

The study was designed to assess the relative tolerance and safety of chlorine dioxide, chlorite, chlorate, chlorine and chloramine ingestion in man. The study was conducted in three parts. Phase I was designed to evaluate the acute physiological effects of single rising dose administration of the disinfectants to normal healthy adult males. Chronic ingestion by normal male volunteers was studied in Phase II. Phase III assessed the physiological response of potentially susceptible individuals to chronic ingestion of chlorite.

MATERIALS AND METHODS

Subject Selection

Normal healthy adult male volunteers selected for inclusion in this study were required to satisfy the following criteria:

1. Subjects were between 21 and 45 years of age and were not confined to an institution;

2. Subjects had no significant abnormal physical findings at the pretreatment physical examination;

3. Subjects had no laboratory values significantly outside the normal range at the pretreatment evaluation;

4. Subjects weighed at the time of the pretreatment evaluation within $\pm$ 10% of the normal body weight for their frame and stature based on a standard weight table;

5. All subjects gave written consent to participate in the study.

Subjects were excluded from the study if they:

1. Had symptoms of significant clinical illness in the two weeks preceding the study;

2. Received any drugs in the four weeks preceding the initiation of this study.

3. Required any concomitant medication;

4. Had one or more surgical or medical condition which might interfere with the absorption, metabolism or excretion of substances by the body;

5. Had a known hypersensitivity to drugs, food or environmental factors;

6. Had a history of cardiac, renal, hepatic, neurologic, hematologic or gastrointestinal disease;

7. Had any abnormal findings on the pretreatment physical examination.

8. Had a history of drug addiction.

For Phase III, volunteers were defined as glucose-6-phosphate dehydrogenase (G-6-PD) deficient on the basis of a hemoglobin G-6-PD level of less than 5.0 IU/GM HB in the pre-study screening. Phase III subjects were normal in all other respects.

Concurrent Drug Therapy

No subject who entered this study was undergoing concurrent drug therapy. However, once on study, a record was kept of the occasional episode of any physician-administered drug therapy that was required because of intercurrent illness. No subject was dropped from the study because of any intercurrent illness that developed during this study.

Institutional Review Committee

This study was not initiated until it was approved by the Human Investigations Committee at The Ohio State University, College of Medicine.

Informed Consent Form

An informed consent form was signed by each participating human subject. A specimen copy of the consent form signed by each participant in this study is presented in the appendix. A copy of each individual consent form for each participant has been kept on file at Ohio State University, College of Medicine. These consent forms are available for inspection, if needed.

Preparation of Disinfectants

The water disinfectant preparations studied were freshly prepared for each study day, labelled, stored and refrigerated in sealed amber glass 500 mL bottles. All bottles were coded by an independent obsever and the identity of each bottle remained "double-blinded" to

both the investigative staff and the volunteer subjects throughout the study. It was necessary to break the individual code for a single subject in Phase III of the study.

Stock Solutions--

Chlorine dioxide (ClO_2)--A chlorine dioxide stock solution was prepared daily. A mixture of potassium chlorate (2 g) and oxalic acid (1 g) was ground to a fine powder. Concentrated sulfuric acid (8 mL) was added dropwise, with cooling, in a darkened room. The gaseous ClO_2 was drawn by vacuum into deionized water.

Chlorine (Cl_2)--Chlorine stock solutions were prepared twice weekly. Commercial chlorine gas bubbled through deionized water until a distinct yellow color was visible.

Chloramine (NH_2Cl)--Chloramine was prepared daily by a modified Schmitz method. Dilute sodium hypochlorite (5% aqueous, 68 mL) added dropwise to ammonium hydroxide (2N) with external cooling. The addition was made very slowly (50-60 min) with vigorous stirring. Vacuum distillation yielded a concentrated solution of chloramine in water. The stock solution was diluted 1:5 with deionized water stored under refrigeration until use.

Chlorite (ClO_2^-) and chlorate (ClO_3^-)--Stock solutions of sodium chlorite and sodium chlorate were made by weight from commercial pure reagent-grade crystals. Assays of the chloramine, chlorine dioxide, chlorine, and chlorite stock solutions were made according to the methods of Palin. Chloramine solutions were assayed for concentrations of monochloramine and dichloramine, and free chlorine. The chlorine dioxide solutions were analyzed for concentrations of chlorine, chlorine dioxide, and chlorite; for the chlorine solutions, chlorine and chlorite concentrations were determined. The Palin method was used to verify the accuracy of the gravimetric concentrations of chlorite solutions. The chlorate solutions were periodically assayed to verify negligible chlorine, chlorine dioxide, chloramine, and chlorite concentrations.

After determining stock solution concentrations, appropriate dilutions were made to yield final solutions of 5 mg/L concentrations.

Chemicals--

The chemicals used in this study were of the highest purity available. The specific sources of each chemical is listed below:

Oxalic acid	(reagent A.C.S. grade, LeHigh Valley Chemical Co.)
Potassium chlorate	(crystalline, J.T. Baker Chemical Co.)
Sulfuric acid	(98%, reagent A.C.S. grade, Fisher Scientific Co.)
Chlorine	(99%, Fisher Scientific Co.)
Sodium chlorate	(reagent, Matheson Coleman & Bell, Inc.)

Sodium chlorite	(reagent, Matheson Coleman & Bell, Inc.)
Sodium chlorite	(flake, practical, Matheson Coleman & Bell, Inc.)
Ferrous ammonium sulfate	(reagent A.C.S., Matheson Coleman & Bell, Inc.)
Malonic acid	(Pfaltz and Bauer, Inc.)
DPD	(#1 powder, LaMotte Chemical)
Potassium iodide	(reagent grade, Scientific Products)
Ammonium hydroxide	(reagent grade, J.T. Baker Chemical Co.)
Sodium hypochlorite	(practical grade, 5.25%, The Clorox Co.)

Study Design

Phase I--

The sixty volunteers of Phase I were divided at random into six treatment groups. Ten persons were assigned to each of the disinfectants or to the control group to receive untreated water.

The study involved a series of six treatment sequences. Treatment concentrations were increased on each treatment day; concentrations administered to the study participants are listed in Table 1. A clinical evaluation and the collection of blood and urine samples for determination of pretreatment baseline laboratory values preceded the first treatment. On the first day of each three day treatment sequence, each volunteer ingested 1000 mL of treated water. All treatments were divided into two 500 mL aliquots; the second aliquot was administered four hours after the first. Each 500 mL portion was consumed within 15 minutes of administration. The second day of the treatment sequence consisted of a physical examination and collection of blood and urine samples for determination of posttreatment laboratory values. On the third day, each volunteer was given a physical examination to determine residual effects of treatment with the water disinfectants and byproducts.

Taste evaluations were obtained at each dose level. Study participants were asked to rate the treated water as very unpleasant, slightly unpleasant, not pleasant, pleasant, or tasteless.

Phase II--

The sixty volunteers of Phase II were divided at random into six treatment groups of ten subjects each. In order to assure efficient management of the 60 subjects, they were randomly assigned to three subsets. These subsets were sequentially entered into the study on three successive days and exited from this study in a similar fashion. For all of the treatment groups, the concentration of disinfectants ingested was 5 mg/L. The control group received untreated water. Each subject received 500 mL daily for twelve weeks. Table 2 lists individual subject assignments. Physicals, collection of blood and urine samples for laboratory assays, and taste evaluations were conducted on a weekly basis during the treatment period and for eight weeks following cessation of treatment.

Phase III--

All subjects in Phase III were given sodium chlorite at a concentration of 5 mg/L chlorite. The treatment protocol was identical to that of Phase II.

Evaluation

The evaluation of response to treatment was multifaceted. It included a medical history, a physical examination, a compilation of vital signs, a side effects record and a battery of laboratory tests.

The forms used for tabulation of the medical histories, physical examinations, vital signs and side effects are included in the appendix.

The schedule of evaluation procedures for Phase I is presented in Table 3. Table 4 gives the schedule of evaluations conducted in Phase II and Phase III.

The specific quantitative assays performed in the analysis of the urine and blood samples are listed in Table 5. Established normal ranges and appropriate abbreviations are included. The assays were performed by Consolidated Biomedical Laboratories, Columbus, Ohio.

Statistical Analysis

The numerical values were collected and analyzed utilizing the facilities of The Ohio State University, Division of Computing Services for Medical Education and Research. Specially designed programs prepared by Jerry Martin and Mike Oskins facilitated rapid clinical feedback. Any value for an individual subject which differed from the group mean by more than two standard deviations was flagged. In addition, every individual value which fell outside normal laboratory ranges for that parameter was designated as abnormal. Chemical parameters for volunteers who exhibited abnormal values were subjected to careful scrutiny; the safety and the possibility of hypersensitivity to the disinfectant agents were evaluated for each of these individuals prior to continued administration of disinfectant.

Statistical analyses utilized commercially available computer packages. Two-way analyses of variance with repeated measures utilized Biomedical Computer Programs-2V (BMDP-2V). BMDP-1R was used to perform multiple linear regression analyses. For pairwise t-tests and simple t-tests, the Statistical Package for the Social Sciences (SPSS-t-test) was employed.

RESULTS

Qualitative Observations

An important aspect of this study was the careful and continued medical observation of all subjects. The general clinical histories

and physical examinations along with subjective observations and qualitative laboratory tests gathered throughout this study were accumulated in each subject's medical file. A careful inspection of each of these medical files presents a review of the general clinical health of each subject. A summary of these clinical results for each subject is found in the appendix.

The careful clinical evaluation of every subject in Phases I, II, and III failed to reveal any clinical important impact upon the medical well-being of any subject as a result of disinfectant ingestion. Further, there was no apparent grouping of the minor subjective symptoms and objective signs noted throughout the study; the "colds", "lymphadenopathy", "sore throats" and "flu" problems noted episodically appear to be randomly dispersed among the treatment groups. All subjects remained negative with respect to the Coombs tests and the sickle cell tests during the investigation. Hemoglobin electrophoresis results yielded a small number of borderline abnormalities in Phase II, but these were evenly distributed among the treatment groups and the control group. Examination of electrocardiograms revealed no abnormalities.

Quantitative Parameters

Vital Signs--

Vital signs (blood pressure, pulse rate, respiration rate and body temperature) were determined as indicated in Tables 3 and 4. The measurement of vital signs was conducted primarily to provide immediate feedback to monitoring physician on the acute physiological response of study participants to treatment. An individual's vital signs provide a supplementary retrospective indication of his overall physical condition and aid in diagnosis of concurrent illnesses. Collective vital signs present the potential for identification of acute physiological response to treatment.

The statistical analysis of the vital signs was limited to the calculation of arithmetic group means and standard deviations from the mean. Tabulations of these results are found in the appropriate appendices. The compiled vital signs were examined for evidence of consistent response to treatment. No such evidence was found.

Biochemical Parameters--

Phase I--Two-way analyses of variance with repeated measures were used to compare the treatment group values of each biochemical parameter to the corresponding values of the control group. The six treatment sequence was treated as a single time-dependent experiment. The use of the analysis of variance allowed distinctions to be made among the possible sources of variation. Intrinsic differences between two groups, parallel variations in quantitative chemical values due to laboratory drift, and authentic treatment-related changes in physiological parameters could be distinguished. Three

probabilities were calculated for each case: the group main effect (G), the time main effect (R), and group-time interaction (RG).

In 121 instances, the comparison of treatment group quantitative biochemical parameters with those of the control group yielded an R-value of less or equal to 0.05. In each case, the corresponding RG- and G-values were large, exceeding 0.05. The occurrence of a statistically significant R-probability in the absence of statistically significant RG- or G-values is most likely indicative of parallel patterns of change with respect to time for the treatment group and the control group. Noting that all laboratory determinations were made in a licensed commercial biomedical facility with stringent quality control, the individual values were examined. Group mean values and the standard deviations from the mean were reviewed. In all cases, the magnitude of variation with time identified as probable by the statistical analyses of variance lay well within accepted operational limits. It was concluded that the parallel patterns of change in the treatment groups and in the control group suggested by small R-probabilities was most likely caused by laboratory drift of small proportion.

The biochemical parameters and treatment groups for which the two-way analysis of variance with repeated measures indicated G- or RG-values of less than 0.05 are tabulated in Table 6.

In five cases, a small G-value ($G \leq 0.05$) was accompanied by a small R-value ($R \leq 0.05$). The treatment groups and chemical tests for which this result was obtained are: the chlorine treatment group for mean corpuscular volume and mean corpuscular hemoglobin, the chlorate treatment group for uric acid and basophils, and the chlorine dioxide-treatment group for lymphocytes. In this study, when a statistically significant group difference ($G \leq 0.05$) is accompanied by a statistically significant variation with respect to time ($R \leq 0.5$) and a nonsignifiant group-time interaction, one can make two statements with reasonable certainty. First, for each of these laboratory parameters, a certain amount of parallel drift in laboratory values within the standards for quality control is observed in both the treatment groups and the control group. Second, for each of these laboratory parameters, there exists a reasonable probability that an intrinsic difference exists between the control group and the treatment group, which is maintained throughout the study.

A G-value of less than or equal to 0.05, in the absence of RG- and R-values of similar proportion is observed for three cases: the glucose-6-phosphate dehydrogenase levels of the chlorine group, the uric acid levels of the chlorite group and the urea nitrogen levels in the chlorate treatment group. One may conclude that the group values of these parameters probably did not vary with respect to time and that a fairly constant difference in value between the treatment group and the control group was maintained during the entire study.

In ten cases, a RG-value of less than or equal to 0.05 was accompanied by a R-value of less than or equal to 0.05. The treatment groups and chemical tests for which this phenomenon was observed are as follows: the chlorine treatment group for calcium, creatinine, lymphocytes, BUN/creatinine ratio and gamma glutamyl transferase, the chlorite treatment group for creatinine, urea nitrogen, and gamma glutamyl transferase, and the chlorate treatment group for bilirubin and serum iron. A significant group-time interaction in the presence of a significant variation with time indicates that the pattern of change with respect to time was different for the control group mean values and the treatment group mean values. The concurrence of small R- and RG-values is best explained by the existence of some source of variation within the treatment group which is not reflected in the corresponding laboratory values of the control group. It can be postulated that the probable source of variation is the physiological response to the disinfectant or disinfectant byproduct administered to the treatment group.

When the methemoglobin values of the chlorate group and the uric acid values of the chlorine dioxide group were compared to the corresponding values of the control group, R- and RG-values of 0.92; 0.02 and 0.25; 0.02 were obtained. These values indicate the presence of a statistically significant group-time interaction without an accompanying statistically significant time main effect. They may be interpreted as examples of physiological responses to treatment in which changes in the laboratory parameters of the treatment group were opposite in magnitude to those of the control group.

The precision of the laboratory determinations of biochemical parameters exceeded the precision of normal physiological regulation of biochemical levels in the human body. Therefore, it must be noted with caution that none of these statistically significant differences in the pattern of change with respect to time is necessarily of physiological consequence (Berg, 1979 and Glantz, 1980).

To assist in determining the clinical importance of the statistically significant group time interactions, the group mean and standard deviations from the mean were examined for the pretreatment baseline assay and each posttreatment assay for each group. These values are listed in Table 7, with the normal ranges for the biochemical parameters. In all instances, group mean values remain well within the established normal ranges.

The unpaired t-test was used to compare treatment group values to the corresponding controls. Two pairs of treatment group-control group values yielded probabilities of less than 0.05 when compared to one another, suggesting significant difference. However, with a P-value equal to 0.05, there is one chance in twenty that the observed difference could have been due to chance. The two small P-values are fewer than would be expected. Consequently, no definitive clinical importance can be attributed to the results of the unpaired t-tests.

On the basis of the t-tests, a consideration of the small magnitude of change within the normal range, the group means with the corresponding standard deviations and the design and duration of the study, it was concluded that the trends identified by the analysis of variance are unlikely to be of clinical importance. The possibility that the trends could achieve proportions of clinical significance with increased study duration could not be evaluated on the basis of this study.

Phase II--Initially, an omnibus testing procedure was employed. To test the hypothesis that the response of one or more of the groups was different to that of the rest of the groups, an analysis of variance with repeated measures was performed. Values for all six treatment groups were included. The results are tabulated in Table 8.

For two parameters, urea nitrogen and mean corpuscular hemoglobin, RG-values of less than 0.05 were obtained. In this case, the small RG-values may be interpreted as indicative of a response to treatment in one or more groups which differs significantly from the response of the other groups.

When the A/G ratios and T-3 (uptake) values were examined by the omnibus test, G-values of less than 0.05 were obtained. On the basis of the analysis of variance, it is reasonable to postulate that at least one of the treatment groups yielded A/G ratios and T-3 (uptake) values consistently different than those of the other groups.

With few exceptions, upon examination by the omnibus test, most parameters yielded statistically significant probabilities for the time main effect (R). In the absence of a small RG-value, the best explanation of this phenomenon is minor parallel fluctuations in values as a result of laboratory drift. An examination of the laboratory parameter values relative to the normal range for each parameter affirmed the small magnitude of the fluctuations.

Supplementary tests were performed to determine which of the treatment groups were responsible for the small RG- and G-values in the omnibus test. Analyses of variance with repeated measures in which the values of each treatment group were compared to the corresponding values of the control group were chosen. Urea nitrogen, A/G ratio, T-3 (uptake) and mean corpuscular hemoglobin values were analyzed. The results are tabulated in Table 9.

The use of the analysis of variance in this manner is flawed by the common control group. However, the results of the analyses may be used with caution.

For urea nitrogen, RG-probabilities of 0.04 and 0.02 were obtained when the chlorate treatment group and the chlorine dioxide treatment group were each compared to the control group. It is reasonable to assume that the urea nitrogen values of both of these groups vary in a different manner than those of the control group.

In the case of mean corpuscular hemoglobin, the analysis of chlorite and chlorate values yielded RG-values of 0.03 and 0.03, respectively. The best explanation of this result is that, in response to ingestion of chlorite or chlorate, MCH values were altered in a different manner than those of the control group.

In T-3 (uptake), a G-value of 0.00 was obtained for the comparison of the chloramine group to the control group. Similarly, the comparison of chlorate group A/G ratios to those of the control group yielded a group main effect (G) values of 0.04. In both instances, a fairly constant difference in values between the treatment group and the control group is likely.

The precision of the laboratory determination of biochemical parameters exceeds the limits of biochemical regulation within the body. Therefore, the values that yielded the statistically significant RG-values in the comparison of the chlorite group and chlorate group mean corpuscular hemoglobin values and the chlorate group and chlorine dioxide group urea nitrogen values to the corresponding control group values must be examined again. This examination must assess the extent of physiological consequence of the dissimilar variation identified as probable by statistical means.

The assumption can be made that a treatment-related change in value may yield a linear change with respect to time. Linear regression analyses were applied to each of the cases which gave small RG-values. Table 10 lists the results.

Mean corpuscular hemoglobin levels in the chlorate group yielded a probability of 0.01 upon linear regression analysis. The relative slope associated with the change during the twelve week treatment period was approximately one percent of the normal physiological range per week. Figure 1 shows the group mean values and the standard deviations. We believe that no physiological importance may be attributed with confidence to the variation. However, it is impossible on the basis of this study to rule out the potential physiological significance of the trend. Further study is warranted.

Since the assumption of linear variation does not necessarily hold, plots were made for the other three cases of small RG-values. Figures 2, 3, and 4 present the group means and standard deviations from the mean for these cases.

Upon examination of these plots, the physiological consequence of the variation seems to be minuscule. However, one cannot negate the possibility that an extended exposure to the disinfectants could produce an effect of clinical importance. Additional study is needed.

Previous studies (Hefferman, 1979 and Abdel-Rahman, 1979), both *in vitro* and *in vivo* animal experimentation, have found physiological impact upon hemoglobin, hematocrit, thyroid function, methemoglobin, and glutathione levels as a result of chronic oral intake of chlorite

and chlorine dioxide. The statistical analyses outlined above revealed no abnormalities in these parameters in our studies in man. Plots were made of the group mean values and the standard deviations of those values for subjective analysis. The plots are included in the appendix. No notable changes in values occurring as a result of treatment were revealed by the careful subjective examination of these plots.

Phase III--The small number of subjects (three) in this phase of the study negated the values of many statistical procedures. Linear regression analyses calculated the probability of a change with respect to time. Graphical displays of individual values were constructed to facilitate subjective evaluation of non-linear variation.

Table 11 lists the biochemical parameters for which a high probability of change with respect to time was calculated. P-values computed by the linear regression analysis are given. To gauge the relative magnitude of change, the percent change of the normal range per week was also computed.

The statistical analyses indicate a good probability that, for A/G ratio, T-4 (RIA), free thyroxine, mean corpuscular hemoglobin concentration, and methemoglobin values, a change with respect to time occurs during the twelve week treatment period. However, in the absence of a concurrent control group and taking into consideration the small group size and the possibility of laboratory drift, one must exercise caution in dealing with the results. We can say with confidence only that trends were indicated. We cannot say that these trends were of physiological origin nor can we attribute physiological consequence to them.

Palatability

The subjective evaluations of taste were recorded after each treatment in Phase I of the study. Taste perceptions were recorded specifically to evaluate taste tolerance to the disinfectants under laboratory conditions in the absence of organic water contaminants. The results of the taste critiques are tabulated in Table 12. As would be expected, individual perceptions of taste varied a great deal within each treatment group. The strongest objections to taste were registered in the chlorine treatment group, with more than half of the members terming the taste of the two highest doses "very unpleasant". The taste of concentrated chlorite and chlorate solutions evoked few strongly negative responses. Levels of negative response for the chloramine and chlorine dioxide solutions were roughly parallel; the 24 ppm level, most of the study participants perceived the taste of the solutions to be unpleasant. The organic-free demineralized deionized water was rated as unpleasant by one-sixth of the control group. The power of suggestion was demonstrated when, as the study progressed and the "concentration" of water was "increased", perceptions of the taste of the water became increasingly negative.

DISCUSSION

During the course of the three phase study, a massive volume of raw data was acquired. For each of the participants, subjective information was collected at regular intervals concerning general medical well-being. The interpretation of physical examinations, electrocardiograms, Coombs tests, and hemoglobin electrophoresis increased the amount of available information. Vital signs (blood pressure, heart rate, body temperature and respiratory rate) for each study subject were determined and compiled. Qualitative examinations of individual blood and urine samples were made. Further, 47 quantitative chemical parameters derived from the extensive battery of blood and urine testing procedures were recorded regularly.

By meticulous examination of this body of information, assessment of the safety of chlorine dioxide and its metabolites was facilitated.

The incidence of minor illnesses, such as "colds" and the "flu" were evenly distributed among treatment groups and control group. Physical examinations revealed no treatment-related pathology. Coombs test results were consistently normal throughout the study for the treatment groups and the control groups. The incidence of borderline abnormal hemoglobin electrophoresis was greatest during the posttreatment observation period; no relationship between treatment and the presence of abnormal hemoglobin electrophoresis could be established. Examination of individual vital sign parameters and statistical evaluation of those parameters failed to identify any changes in vital signs which could be attributed to treatment with any of the water disinfectants. Abnormalities in the qualitative blood and urine analyses were few and, again, appeared to be randomly distributed.

No definitive assessment of detrimental physiological impact was made in any of the three phases of this human investigation of the relative safety and tolerance of oral chlorine disinfectant ingestion. In several cases, statistically significant trends were associated with treatment; however, none of these trends were judged to have physiological consequence. One cannot rule out the possibility that, over a longer treatment period, these trends might indeed achieve proportions of clinical importance. However, within the limits of the study, the relative safety of oral ingestion of chlorine dioxide and its metabolites, chlorite and chlorate, was demonstrated.

BIBLIOGRAPHY

Abdel-Rahman, M.S., Couri, D., and Bull, R.J.: Kinetics of ClO_2 and effects of ClO_2, ClO_2^-, and ClO_3^- in drinking water on blood glutathione and hemolysis in rat and chicken. J. Env. Path. Toxicol.3 (1,2): 431-449, 1980.

Berg, A.O.: Some non-random views of statistical significance. J. Family Practice 8: 1011-1014, 1979.

Couri, D. and Abdel-Rahman, M.S.: Effect of chlorine dioxide and metabolites on glutathione dependent system in rat, mouse and chicken blood. J. Env. Path. Toxicol. 3 (1,2): 451-460, 1980.

Enger, M.: Treatment of water with chlorine dioxide to improve taste. Gas and Wasser Fach 14: 340-343, 1960.

Fridlyand, S.A. and Kagan, G.Z.: Experimental validation of standards for residual chlorine dioxide in drinking water. Hygiene Sanitation 36: 18-21, 1971.

Glantz, S.A.: Biostatistics: how to detect, correct, and prevent errors in the medical literature. Circulation 61: 1-6, 1980.

Haller, S.F. and Northgraves, W.W.: Chlorine dioxide and safety. TAPP, 33 No. 4, 199-203, 1955.

Hefferman, W.P., Guion, C. and Bull, R.J.: Oxidative damage to the erythrocyte induced by sodium chlorite *in vivo*. J. Env. Path. Toxicol. 2(6): 1487-1499, 1979.

Hefferman, W.P., Guion, C. and Bull, R.J.: Oxidative damage to the erythrocyte induced by sodium chlorite *in vitro*. J. Env. Path. Toxicol. 2(6): 1501-1510, 1979.

Jung, F. and Kuon, R.: Zum inaktiven hemoglobin das bluter. Naunyn-Ackmiedebergs. Arch. Exp. Pathol. Pharmakol. 216: 103-111, 1951.

Marx, J.L.: Drinking Water. Another source of carcinogens. Science. 186: 809, 1974.

Miltner, R.: The effect of chlorine dioxide on trihalomethanes in drinking water. University of Cincinnati, Master Thesis, 1976.

Musil, J., Kontek, Z., Chalupa, J. and Schmidt, P.: Toxicological aspects of chlorine dioxide application for the treatment of water containing phenol. Chem Technol. Praze. 8: 327-345, 1964.

Palin, A.T.: Methods for the determination, in water, of free combined available chlorine, chlorine dioxide and chlorite, bromine, iodine and ozone, using diethyl-p-phenylene diamine (DPD). J. Inst. Water Engr. 21: 537, 1967.

Richardson, A.P.: Toxic potentialities of continued administration of chlorate on blood and tissues. J. Pharmacol. and Exper. Therap. 59: 101-110, 1937.

Rook, J.J.: Formation of haloforms during chlorination of natural waters. Water Treat. Exam. 23: 234, 1974.

Schmitz, V.E., Schramm, S., Flamme, W. and Bicker, U.: Nebenreaktionen der chloraminherstellung. Z. Anorg. Allg. Chem. 396: 178-184, 1973.

Stevens, A., Seeger, D. and Slocum, C.J.: Products of chlorine dioxide treatment of organic material in water. Presented at workshop on ozone chlorine dioxide oxidation products of organic materials. Cincinnati, Ohio, November 17-19, 1976.

TABLE 1. CONCENTRATION OF DISINFECTANTS AND SUBJECT ASSIGNMENT (mg/L)
PHASE I: RISING DOSE TOLERANCE STUDY

Water disinfectant	Subject numbers	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
Chlorate	0-9	.01	.1	.5	1.0	1.8	2.4
Untreated water	10-19	0	0	0	0	0	0
Chlorine dioxide	20-29	.1	1.0	5.0	10.0	18.0	24.0
Chlorite	30-39	.01	.1	.5	1.0	1.8	2.4
Chlorine	40-49	.1	1.0	5.0	10.0	18.0	24.0
Chloramine	50-59	.01	1.0	8.0	18.0	24.0	-

TABLE 2. CONCENTRATION OF DISINFECTANTS AND SUBJECT ASSIGNMENT*
PHASE II: CHRONIC ADMINISTRATION TO NORMAL SUBJECTS

Water disinfectant	Group #	Subject numbers
Chlorite, 5 mg/L	1	1,7,13,19,25,31,37,43,49,55
Chlorate, 5 mg/L	2	2,8,14,20,26,32,38,44,50,56
Chlorine, 5 mg/L	3	3,9,15,21,27,33,39,45,51,57
Chlorine dioxide, 5 mg/L	4	4,10,16,22,28,34,40,46,52,58
Chloramine, 5 mg/L	5	5,11,17,23,29,35,41,47,53,59
Untreated water	6	6,12,18,24,30,36,42,48,54,60

* Each subject received a single daily dose of 500 mL of the respective disinfectant or control substance. All doses were prepared in deionized water.

TABLE 3. SCHEDULE FOR EVALUATION PROCEDURES
PHASE I: RISING-DOSE TOLERANCE STUDY

Procedure	Screen	Days 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Dose administration			X			X			X			X			X			X	
Medical history		X																	
Physical examination		X		X	X		X	X		X	X		X	X		X	X		X
Vital signs		X	X	X		X	X		X	X		X	X		X	X		X	X
Subjective evaluation	X		X	X		X	X		X	X		X	X		X	X		X	X
Electrocardiogram	X		X	X			X			X			X			X			X
Lab assay of blood & urine samples	X		X	X			X			X			X			X			X
G-6-PD	X		X							X						X			X
Sickle cell preparation	X		X																X
Hemoglobin electrophoresis	X		X																X
Glutathione	X		X																X

TABLE 4. SCHEDULE FOR EVALUATION PROCEDURES
PHASE II AND PHASE III: CHRONIC STUDIES

Evaluation procedure	Screen	Base-line	Week of study 1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	20
Medical history		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Subjective evaluation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Electrocardio-gram	X	X	X	X		X		X		X		X		X		X	X	X
Lab assay of blood & urine samples	X	X	X	X		X		X		X		X		X		X	X	X
G-6-PD	X	X				X				X				X		X		X
Sickle cell preparation	X	X								X				X				
Hemoglobin electro-phoresis	X	X		X		X				X				X		X		X
Coombs test	X	X	X			X				X		X		X		X		X
Haptoglobin	X	X	X			X				X		X		X		X		X

TABLE 5. BATTERY OF LABORATORY TESTS AND NORMAL RANGES

A. SERUM CHEMISTRY

Tests	Norman ranges
Glucose, plasma	75-120 MG/DL
Sodium	136-146 MEQ/L
Potassium	3.5-5.5 MEQ/L
Chloride	97-108 MEQ/L
Urea nitrogen	10-20 MG/DL
Creatinine	0.7-1.5 MG/DL
BUN/creatinine ratio	8-21
Uric acid	4.0-8.5 MG/DL
Calcium	9.1-10.9 MG/DL
Phosphorus	2.5-4.5 MG/DL
Alkaline phosphatase (ALK PHOS)	30-125 I.U./L
Gamma glutamyl transferase (GGT)	0-65 I.U./L
Bilirubin, Total	0.0-1.3 MG/DL
Serum glutamic-oxaloacetic transaminase (SGOT)	0-42 I.U./L
Serum glutamic-pyruvic transaminase (SGPT)	0-45 I.U./L
Lactic dehydrogenase (LDH)	75-225 I.U./L
Cholesterol	150-300 MG/DL
Triglycerides	30-175 MG/DL
Protein, Total	6.0-8.0 G/DL

(continued)

TABLE 5 (continued)

Tests	Normal ranges
Albumin	3.0-5.0 G/DL
Globulin	1.5-3.4 G/DL
A/G ratio	1.1:1-2.5:1
Iron	40-170 G/DL
T-3 (uptake)	31%-45%
T-4 (RIA)	4.5-12.0 G/DL
Free thyroxine index	1.57-5.40
B. ROUTINE URINALYSIS	
Color	Yellow
Appearance	Clear
Specific gravity	1.010-1.030
pH	5.5-7.5
Protein	0
Sugar	0
Acetone	0
Blood	0
White blood count	0-3
Red blood count	0-3
Casts	0

(continued)

TABLE 5 (continued)

Tests	Normal ranges
Crystals	Few
Bacteria	Few
Mucus	0-1
Amorphous	Few
Epithelial cells	Few
C. COMPLETE BLOOD COUNTS	
Platelet count	150,000-400,000
Blood cell counts	
White blood count (WBC)	4,500-11,000/CMM
Red blood count (RBC)	4.6-6.2 M/CMM
Hemoglobin (HGB)	14-18 GM%
Hematocrit (HCT)	42-52 %
Mean corpuscular volume (MCV)	80-94 C
Mean corpuscular hemoglobin (MCH)	27-31 G
Mean corpuscular hemoglobin concentration (MCHC)	32-36 %
By absolute number	
High peroxidase activity	0-400/CMM
Total neutrophils	1450-7610/CMM

(continued)

TABLE 5 (continued)

Tests	Normal ranges
Lymphocytes	800-2800/CMM
Monocytes	0-760/CMM
Eosinophils	0-380/CMM
Basophils	0-110/CMM
Large unstained cells	0-290/CMM
D. SPECIAL TESTS	
Haptoglobin, Serum	27-139 MG/DL
Sickle cell preparation	Negative
Glutathione	29.3-43.8 MG/DL
Methemoglobin	0.0-0.50 GM/DL
Glucose-6-phosphate dehydrogenase (G-6-PD)	5-10 IU/GM HB
Coombs test direct	Negative
Coombs test indirect	Negative
Hemoglobin electrophoresis	Normal

TABLE 6. TWO-WAY ANALYSIS OF VARIANCE WITH REPEATED MEASURES COMPARISON OF TREATMENT GROUPS TO THE CONTROL GROUP

PHASE I: RISING DOSE TOLERANCE STUDY

Treatment group	Test	Group main effect (G)		Time main effect (R)		Group-time interaction (RG)	
		F-ratio	Probability	F-ratio	Probability	F-ratio	Probability
Chlorite	Urea nitrogen	0.23	0.63	2.31	0.04	2.57	0.02
	Uric acid	5.08	0.04	1.48	0.19	1.64	0.14
	Creatinine	1.02	0.33	5.72	0.00	2.19	0.05
	BUN/creatinine ratio	0.00	0.97	3.67	0.00	2.48	0.03
	Gamma glutamyl transferase	0.01	0.93	10.52	0.00	2.41	0.03
Chlorate	Urea nitrogen	5.14	0.04	2.00	0.07	0.73	0.63
	Uric acid	4.61	0.05	2.63	0.02	1.36	0.24
	Bilirubin, Total	0.29	0.60	12.57	0.00	3.14	0.01
	Iron	0.27	0.61	4.92	0.00	2.71	0.02
	Basophils	7.37	0.01	3.89	0.00	0.75	0.61
	Methemoglobin ($X10^{-1}$)	3.70	0.07	1.87	0.09	2.57	0.02
Chlorine dioxide	Uric acid	1.35	0.26	1.33	0.25	2.67	0.02
	Lymphocytes	6.14	0.02	17.97	0.00	1.47	0.20

(continued)

TABLE 6 (continued)

Treatment group	Test	Group main effect (G)		Time main effect (R)		Group-time interaction (RG)	
		F-ratio	Probability	F-ratio	Probability	F-ratio	Probability
Chlorine	Calcium	0.07	0.80	7.09	0.00	2.44	0.03
	Creatinine	0.05	0.83	2.90	0.01	2.64	0.02
	Gamma glutamyl transferase	0.50	0.49	8.57	0.00	3.40	0.00
	Mean corpuscular volume	5.28	0.03	5.33	0.00	0.35	0.91
	Mean corpuscular Hemoglobin	7.53	0.01	11.56	0.00	0.64	0.70
	Lymphocytes	0.42	0.53	15.10	0.00	2.25	0.04
	Glucose-6-phosphate dehydrogenase	4.55	0.05	1.36	0.19	2.02	0.07

TABLE 7. SELECTED GROUP MEAN VALUES AND STANDARD DEVIATIONS FROM THE MEAN

PHASE I: RISING DOSE TOLERANCE STUDY

Parameter normal range*	Treatment group	Pretreatment	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Urea nitrogen (BUN) 10-20 MG/L	Control	15 ± 3	14 ± 3	15 ± 3	13 ± 4	13 ± 3	14 ± 3	14 ± 5
	Chlorite	15 ± 3	14 ± 4	13 ± 2	15 ± 4	12 ± 3	13 ± 2	12 ± 2
Creatinine 0.7-1.5 MG/DL	Control	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
	Chlorite	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
	Chlorine dioxide	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
BUN/creatinine ratio 8-21	Control	13.1 ± 3.4	13.1 ± 2.8	12.4 ± 2.0	11.6 ± 2.4	11.8 ± 2.6	12.8 ± 2.5	11.8 ± 2.4
	Chlorite	13.3 ± 2.4	13.9 ± 3.5	11.6 ± 2.5	13.4 ± 2.7	10.7 ± 2.8	11.5 ± 1.8	11.5 ± 1.7
Gamma glutamyl transferase 0-65 IU/L	Control	17 ± 11	17 ± 8	13 ± 6	17 ± 5	10 ± 7	15 ± 8	15 ± 8
	Chlorite	14 ± 5	13 ± 7	13 ± 9	14 ± 8	10 ± 9	13 ± 9	18 ± 9
	Chlorine dioxide	19 ± 8	18 ± 18	18 ± 23	19 ± 18	15 ± 17	17 ± 18	18 ± 19
Uric acid 4.0-8.5 MG/DL	Control	5.3 ± 0.7†	5.6 ± 0.8	5.5 ± 0.8	5.2 ± 0.5	5.8 ± 0.6	5.4 ± 0.8	5.3 ± 0.5
	Chlorine dioxide	6.1 ± 0.9†	5.8 ± 0.5	5.5 ± 0.9	5.6 ± 0.5	5.6 ± 0.6	5.6 ± 0.4	5.7 ± 0.8
Bilirubin 0.0-1.3 MG/DL	Control	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
	Chlorate	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2

(continued)

*Normals determined by Consolidated Biomedical Laboratories, Inc. H.E.W. License #34-1030

†Values yielded $P \leq 0.05$ when analyzed by the simple t-test comparing parallel control group and treatment group parameters

TABLE 7 (continued)

Parameter normal range*	Treatment group	Pretreatment	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
Iron 40-170 G/DL	Control	98 ± 36	101 ± 43	95 ± 30	87 ± 29	89 ± 27	91 ± 28†	93 ± 20
	Chlorate	130 ± 48	129 ± 39	102 ± 22	98 ± 29	79 ± 11	67 ± 15†	85 ± 14
Methemoglobin 0.0-0.5 GM/DL	Control	0.027 ± 0.03	0.04 ± 0.05	0.11 ± 0.05	0.09 ± 0.05	0.07 ± 0.08	0.05 ± 0.03	0.05 ± 0.03
	Chlorate	0.10 ± 0.06	0.12 ± 0.08	0.06 ± 0.07	0.11 ± 0.05	0.06 ± 0.05	0.08 ± 0.05	0.05 ± 0.03
Calcium 9.1-10.9 MG/DL	Control	10.1 ± 0.4	10.0 ± 0.2	10.0 ± 0.2	9.8 ± 0.2	9.7 ± 0.3	9.9 ± 0.3	9.8 ± 0.2
	Chlorine	10.1 ± 0.2	10.0 ± 0.3	9.7 ± 0.4	9.8 ± 0.2	9.7 ± 0.2	10.0 ± 0.4	9.8 ± 0.3
Lymphocytes 800-2800 cells/CMM	Control	2830 ± 510	2240 ± 350	2540 ± 580	2350 ± 540	2330 ± 410	2000 ± 520	1900 ± 430
	Chlorine	2540 ± 420	2290 ± 410	2190 ± 430	2010 ± 470	2140 ± 540	2160 ± 450	1830 ± 510

*Normals determined by Consolidated Biomedical Laboratories, Inc. H.E.W. License #34-1030
†Values yielded $P \leq 0.05$ when analyzed by the simple t-test comparing parallel control group and treatment group parameters

TABLE 8. OMNIBUS TEST
ANALYSIS OF VARIANCE WITH REPEATED MEASURES
(all six groups)

PHASE II: CHRONIC ADMINISTRATION TO NORMAL VOLUNTEERS

	Probabilities*		
	Group main effect (G)	Time main effect (R)	Group-time interaction (RG)
Glucose, plasma	0.94	0.00	0.87
Sodium	0.06	0.00	0.96
Potassium	0.95	0.01	0.79
Chloride	0.08	0.00	0.90
Urea nitrogen	0.25	0.23	0.01
Creatinine	0.63	0.00	0.84
BUN/creatinine ratio	0.30	0.00	0.13
Uric acid	0.32	0.00	0.07
Calcium	0.29	0.00	0.75
Phosphorus	0.36	0.04	0.36
Alkaline phosphatase	0.85	0.00	0.73
Gamma glutamyl transferase	0.70	0.00	0.80
Bilirubin, Total	0.79	0.00	0.59
Serum glutamic-oxaloacetic transaminase	0.82	0.00	0.87

* G = Group main effect; R = time main effect; RG = group time interaction

† Probability < 0.05

(continued)

TABLE 8. (continued)

	Probabilities*		
	Group main effect (G)	Time main effect (R)	Group-time interaction (RG)
Serum glutamic-pyruvic transaminase	0.99	0.01	0.95
Lactic dehydrogenase	0.24	0.00	0.39
Cholesterol	0.48	0.00	0.69
Triglycerides	0.90	0.00	0.19
Protein, Total	0.61	0.00	0.98
Albumin	0.56	0.00	0.91
Globulin	0.09	0.00	1.00
A/G ratio	0.04†	0.00	0.98
Iron	0.66	0.02	0.19
T-3 (uptake)	0.02†	0.00	1.00
T-4 (RIA)	0.58	0.00	1.00
Free thyroxine index	0.81	0.00	1.00
Platelet count	0.23	0.00	0.38
White blood count	0.87	0.11	0.33
Red blood count	0.97	0.05	0.66
Hemoglobin	0.64	0.19	0.98

* G = Group main effect; R = time main effect; RG = group time interaction

† Probability $\leq$ 0.05

(continued)

TABLE 8. (continued)

	Probabilities*		
	Group main effect (G)	Time main effect (R)	Group-time interaction (RG)
Hematocrit	0.72	0.04	0.44
Mean corpuscular volume	0.24	0.04	0.30
Mean corpuscular hemoglobin	0.37	0.00	0.04†
Mean corpuscular hemoglobin concentration	0.66	0.00	0.04
High peroxidase activity	0.61	0.00	0.66
Total neutrophils	0.89	0.00	0.17
Lymphocytes	0.45	0.00	0.14
Monocytes	0.49	0.00	1.00
Eosinophils	0.66	0.00	0.77
Basophils	0.53	0.00	0.68
Large unstained cells	0.19	0.00	0.42
Haptoglobin, S	0.96	0.09	0.28
Urine, Sp. gravity	0.19	0.37	0.07
Urine, pH	0.15	0.00	0.36
Methemoglobin ($X10^{-1}$)	0.63	0.00	0.98

* G = Group main effect; R = time main effect; RG = group time interaction

† Probability $\leq$ 0.05

(continued)

TABLE 8. (continued)

	Probabilities*		
	Group main effect (G)	Time main effect (R)	Group-time interaction (RG)
Glucose-6-phosphate dehydrogenase	0.96	0.10	0.28
Glutathione	0.89	0.00	0.99

* G = Group main effect; R = time main effect; RG = group time interaction

† Probability $\leq$ 0.05

TABLE 9. ANALYSIS OF VARIANCE WITH REPEATED MEASURES
(Treatment group vs control)

PHASE II: CHRONIC ADMINISTRATION TO NORMAL VOLUNTEERS

Biochemical parameter	Treatment group	Probabilities		
		Group main effect (G)	Time main effect (R)	Group-time interaction (RG)
Urea nitrogen	Chlorite	0.12	1.00	0.19
	Chlorate	0.03	0.02	0.04
	Chlorine	0.14	0.05	0.58
	Chlorine dioxide	0.32	0.21	0.02
	Chloramine	0.02	0.43	0.97
A/G ratio	Chlorite	0.59	0.00	0.26
	Chlorate	0.04	0.00	0.97
	Chlorine	0.81	0.00	0.81
	Chlorine dioxide	0.97	0.00	0.62
	Chloramine	0.88	0.00	0.80
T-3 uptake	Chlorite	0.29	0.00	0.69
	Chlorate	0.19	0.00	0.83
	Chlorine	0.53	0.00	0.87
	Chlorine dioxide	0.20	0.00	0.99

(continued)

TABLE 9. (continued)

Biochemical parameter	Treatment group	Probabilities		
		Group main effect (G)	Time main effect (R)	Group-time interaction (RG)
T-3 uptake	Chloramine	0.00	0.00	0.87
Mean corpuscular hemoglobin	Chlorite	0.54	0.00	0.03
	Chlorate	0.34	0.03	0.03
	Chlorine	0.84	0.00	0.67
	Chlorine dioxide	0.35	0.00	0.62
	Chloramine	0.41	0.00	0.37

TABLE 10. LINEAR REGRESSION ANALYSIS

PHASE II: CHRONIC ADMINISTRATION TO NORMAL VOLUNTEERS

Treatment groups	Chemical test	Probability	Absolute slope	Normal[§] range		Relative[†] slope
Chlorite vs control*	MCH[#]	0.49	0.021	27-31	G	
Chlorate vs control*	MCH	0.01	-0.038	27-31	G	-0.95%/week
Chlorine dioxide vs control*	Urea nitrogen	0.21	0.097	10-20	MG/L	
Chlorate vs control*	Urea nitrogen	0.55	-0.050	10-20	MG/L	

* The control group was given untreated demineralized deionized water.

† Relative slope was computed from the absolute slope as the % change relative to the normal range.

§ Normal ranges were determined by CBL Laboratories, Inc.

Mean corpuscular hemoglobin

TABLE 11. LINEAR REGRESSION ANALYSIS

PHASE III: CHRONIC ADMINISTRATION OF CHLORITE TO G-6-PD DEFICIENT SUBJECTS

Biochemical parameter	P-value	Absolute slope	Normal* range	Relative† slope
A/G ratio	0.01	+ 0.007	1.1:1-2.5:1	+ 0.5%/week
T-4 (RIA)	0.03	- 0.015	4.5-12.0 G/DL	- 0.2%/week
Free thyroxine	0.01	- 0.036	1.57-5.40	- 0.9%/week
Mean corpuscular hemoglobin concentration	0.00	+ 0.074	32-36%	+ 1.8%/week
Methemoglobin	0.02	- 0.014	0.0-0.5 GM/DL	- 0.3%/week

* Normal ranges as determined by the testing laboratory, Consolidated Biomedical Laboratories, Columbus, Ohio.

† Relative slopes were computed as percent change of the normal physiological range per week.

TABLE 12. PALATABILITY

PHASE I: RISING-DOSE TOLERANCE STUDY

	Very unpleasant	Slightly unpleasant	Not unpleasant	Pleasant	No taste
CHLORATE					
0.01 PPM	1	0	2	0	7
0.10 PPM	0	2	1	1	6
0.50 PPM	1	0	2	0	7
1.00 PPM	1	1	1	1	6
1.80 PPM	2	1	2	1	4
2.40 PPM	1	2	2	0	4
CHLORINE DIOXIDE					
0.10 PPM	0	2	2	0	4
1.00 PPM	0	3	3	1	3
5.00 PPM	0	3	0	0	7
10.00 PPM	1	3	3	0	3
18.00 PPM	2	3	3	0	2
24.00 PPM	2	5	2	0	0
CHLORITE					
0.01 PPM	0	1	1	1	6
0.10 PPM	0	0	1	1	7
0.50 PPM	0	2	1	0	6
1.00 PPM	0	2	1	0	6
1.80 PPM	0	1	1	2	5
2.40 PPM	0	2	1	2	4
CHLORINE					
0.10 PPM	0	0	2	1	6
1.00 PPM	0	4	0	1	4
5.00 PPM	1	2	2	0	4
10.00 PPM	3	4	1	0	1
18.00 PPM	6	2	0	0	1
24.00 PPM	5	3	0	0	0

(continued)

TABLE 12. (continued)

	Very unpleasant	Slightly unpleasant	Not unpleasant	Pleasant	No taste
CHLORAMINE					
0.01 PPM	0	2	3	3	2
1.00 PPM	0	1	5	1	3
8.00 PPM	0	5	2	1	2
18.00 PPM	0	4	4	0	2
24.00 PPM	2	6	1	0	1
WATER	1	9	4	6	38

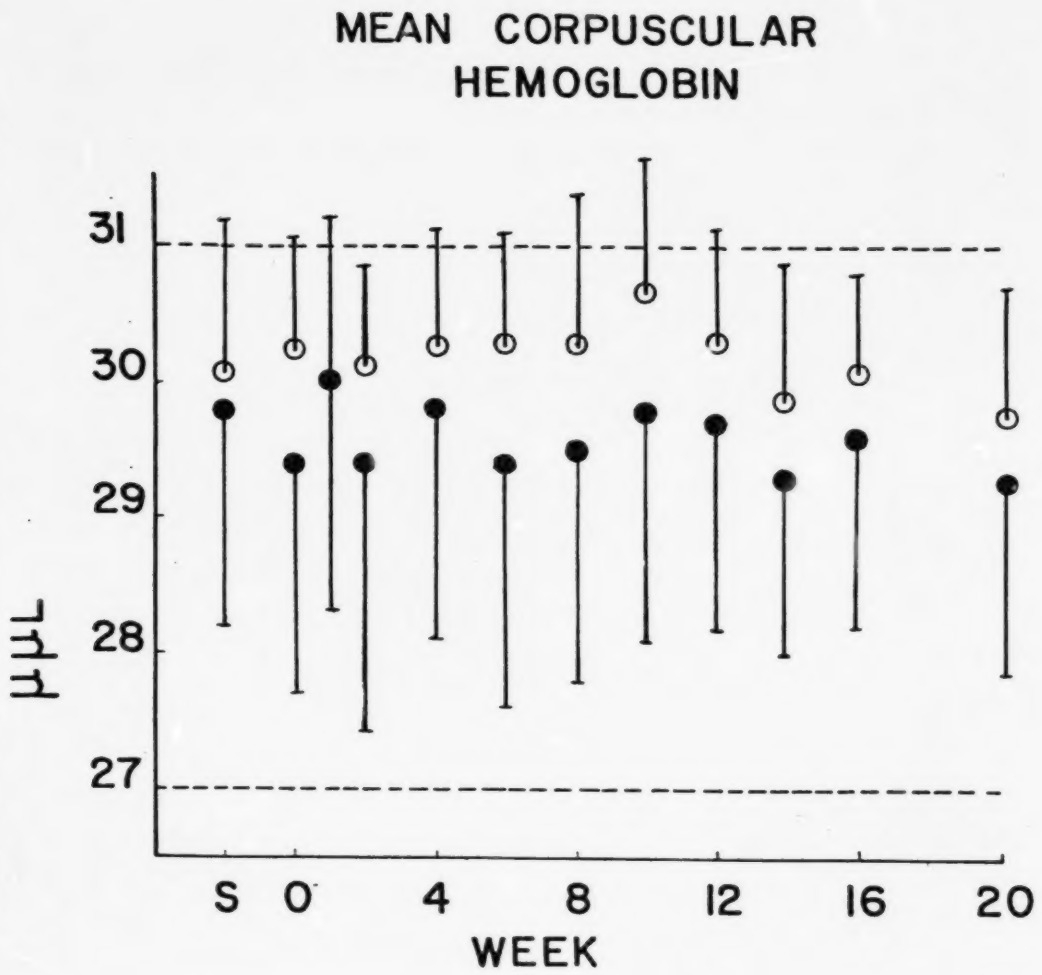


Figure 1. Group arithmetic mean values of mean corpuscular hemoglobin for the chlorate group (○) and the control group (●) and the normal physiological range (---) for the chronic administration of chlorine disinfectants to normal healthy adult volunteers. S = screen test, B = baseline pretreatment assay. Weeks 1 through 12 constitute the treatment period; 13 through 20, the posttreatment observation period (Phase II).

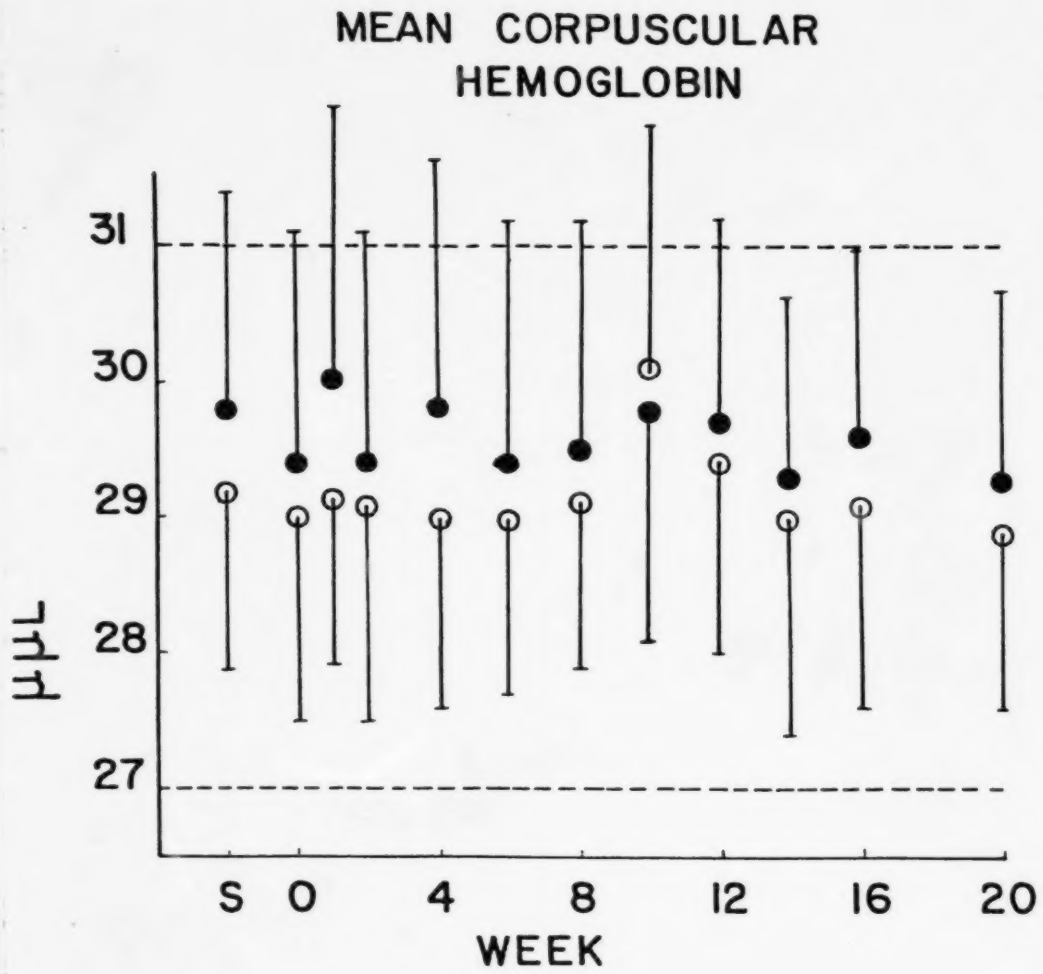


Figure 2. Group arithmetic mean values of mean corpuscular hemoglobin for the chlorite group (○) and the control group (●) and the normal physiological range (---) for the chronic administration of chlorine disinfectants to normal healthy adult volunteers. S = screen test, B = baseline pretreatment assay. Weeks 1 through 12 constitute the treatment period; 13 through 20, the posttreatment observation period (Phase II).

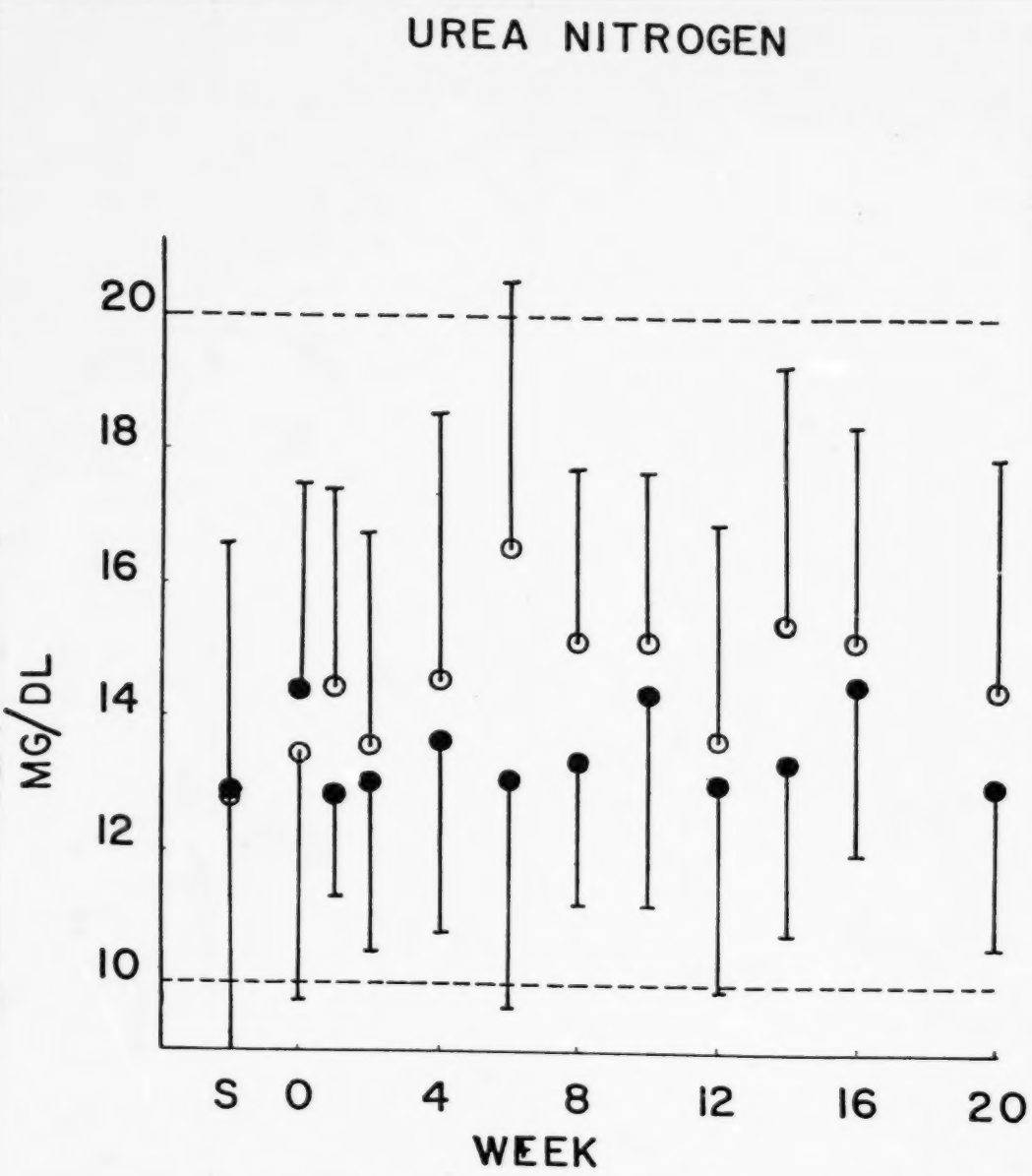


Figure 3. Group arithmetic mean values of urea nitrogen for the chlorine dioxide group (○) and the control group (●) and the normal physiological range (---) for the chronic administration of chlorine disinfectants to normal healthy adult volunteers. S = screen test, B = baseline pretreatment assay. Weeks 1 through 12 constitute the treatment period; 13 through 20, the posttreatment observation period (Phase II).

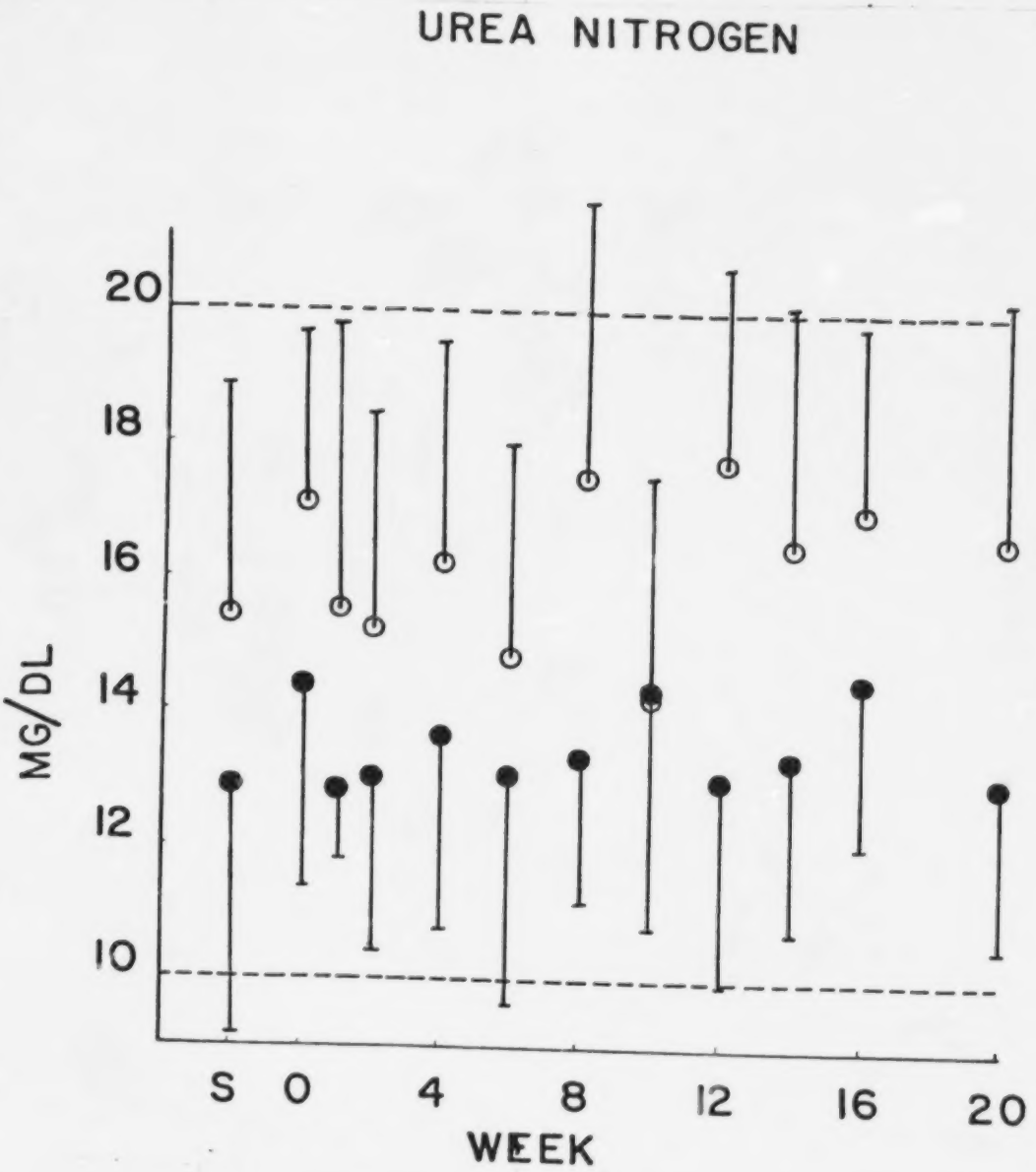


Figure 4. Group arithmetic mean values of urea nitrogen for the chlorate group (○) and the control group (●) and the normal physiological range (---) for the chronic administration of chlorine disinfectants to normal healthy adult volunteers. S = screen test, B = baseline pretreatment assay. Weeks 1 through 12 constitute the treatment period; 13 through 20, the posttreatment observation period (Phase II).

SUPPLEMENTARY FIGURE APPENDIX

Page

Glutathione group means chlorite vs control (Phase II)...1a..... 46
Glutathione group means chlorate vs control (Phase II)...1b..... 47
Glutathione group means chlorine vs control (Phase II)...1c..... 48
Glutathione group means chlorine dioxide vs
control (Phase II).....1d..... 49
Glutathione group means chloramine vs
control (Phase II).....1e..... 50
Hematocrit group means chlorite vs control (Phase II)...2a..... 51
Hematocrit group means chlorate vs control (Phase II)...2b..... 52
Hematocrit group means chlorine vs control (Phase II)...2c..... 53
Hematocrit group means chlorine dioxide vs
control (Phase II).....2d..... 54
Hematocrit group means chloramine vs control (Phase II)..2e..... 55
Red blood count group means chlorite vs
control (Phase II).....3a..... 56
Red blood count group means chlorate vs
control (Phase II).....3b..... 57
Red blood count group means chlorine vs
control (Phase II).....3c..... 58
Red blood count group means chlorine
dioxide vs control (Phase II).....3d..... 59
Red blood count group means chloramine vs
control (Phase II).....3e..... 60
Hemoglobin group means chlorite vs control (Phase II)...4a..... 61
Hemoglobin group means chlorate vs control (Phase II)...4b..... 62
Hemoglobin group means chlorine vs control (Phase II)...4c..... 63
Hemoglobin group means chlorine dioxide
vs control (Phase II).....4d..... 64
Hemoglobin group means chloramine vs control (Phase II)..4e..... 65
T-3 (uptake) group means chlorite vs
control (Phase II).....5a..... 66
T-3 (uptake) group means chlorate vs
control (Phase II).....5b..... 67
T-3 (uptake) group means chlorine vs
control (Phase II).....5c..... 68
T-3 (uptake) group means chlorine dioxide
vs control (Phase II).....5d..... 69
T-3 (uptake) group means chloramine
vs control (Phase II).....5e..... 70

(continued)

Page

Urea nitrogen group means chlorite vs control (Phase II).....6a..... 71
Urea nitrogen group means chlorate vs control (Phase II).....6b..... 72
Urea nitrogen group means chlorine vs control (Phase II).....6c..... 73
Urea nitrogen group means chlorine dioxide vs control (Phase II).....6d..... 74
Urea nitrogen group means chloramine vs control (Phase II).....6e..... 75
Mean corpuscular hemoglobin group means chlorite vs control (Phase II).....7a..... 76
Mean corpuscular hemoglobin group means chlorate vs control (Phase II).....7b..... 77
Mean corpuscular hemoglobin group means chlorine vs control (Phase II).....7c..... 78
Mean corpuscular hemoglobin group means chlorine dioxide vs control (Phase II).....7d..... 79
Mean corpuscular hemoglobin group means chloramine vs control (Phase II).....7e..... 80
A/G ratio group means chlorite vs control (Phase II)...8a..... 81
A/G ratio group means chlorate vs control (Phase II)...8b..... 82
A/G ratio group means chlorine vs control (Phase II)...8c..... 83
A/G ratio group means chlorine dioxide vs control (Phase II).....8d..... 84
A/G ratio group means chloramine vs control (Phase II)...8e..... 85
Methemoglobin group means chlorite vs control (Phase II)...9a..... 86
Methemoglobin group means chlorate vs control (Phase II)...9b..... 87
Methemoglobin group means chlorine vs control (Phase II)...9c..... 88
Methemoglobin group means chlorine dioxide vs control (Phase II).....9d..... 89
Methemoglobin group means chloramine vs control (Phase II).....9e..... 90

FIGURE 1a. GLUTATHIONE

Normal range ----

Control group ●

Chlorite group ○

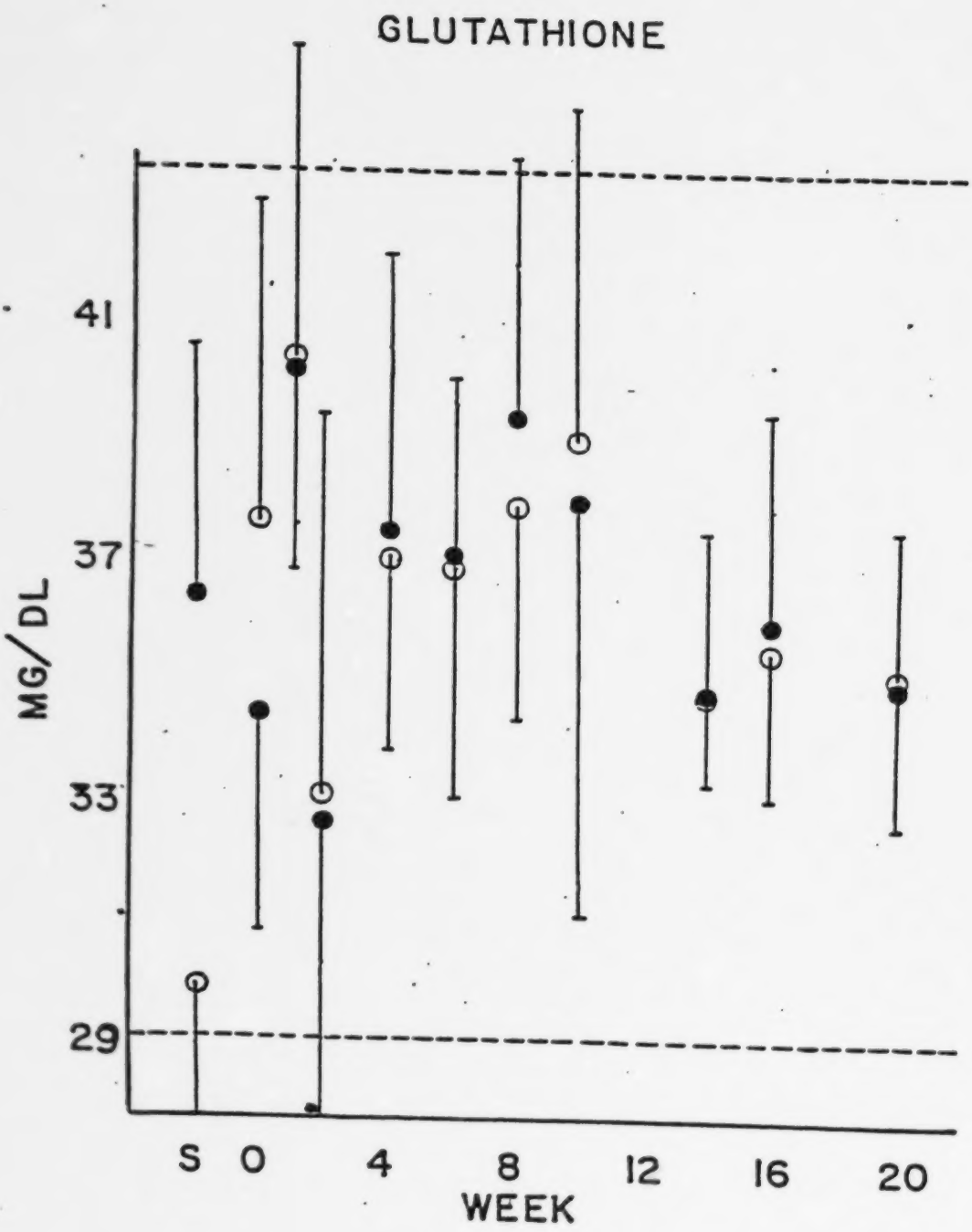


FIGURE 1b. GLUTATHIONE

Normal range ----
Control group ●
Chlorate group ○

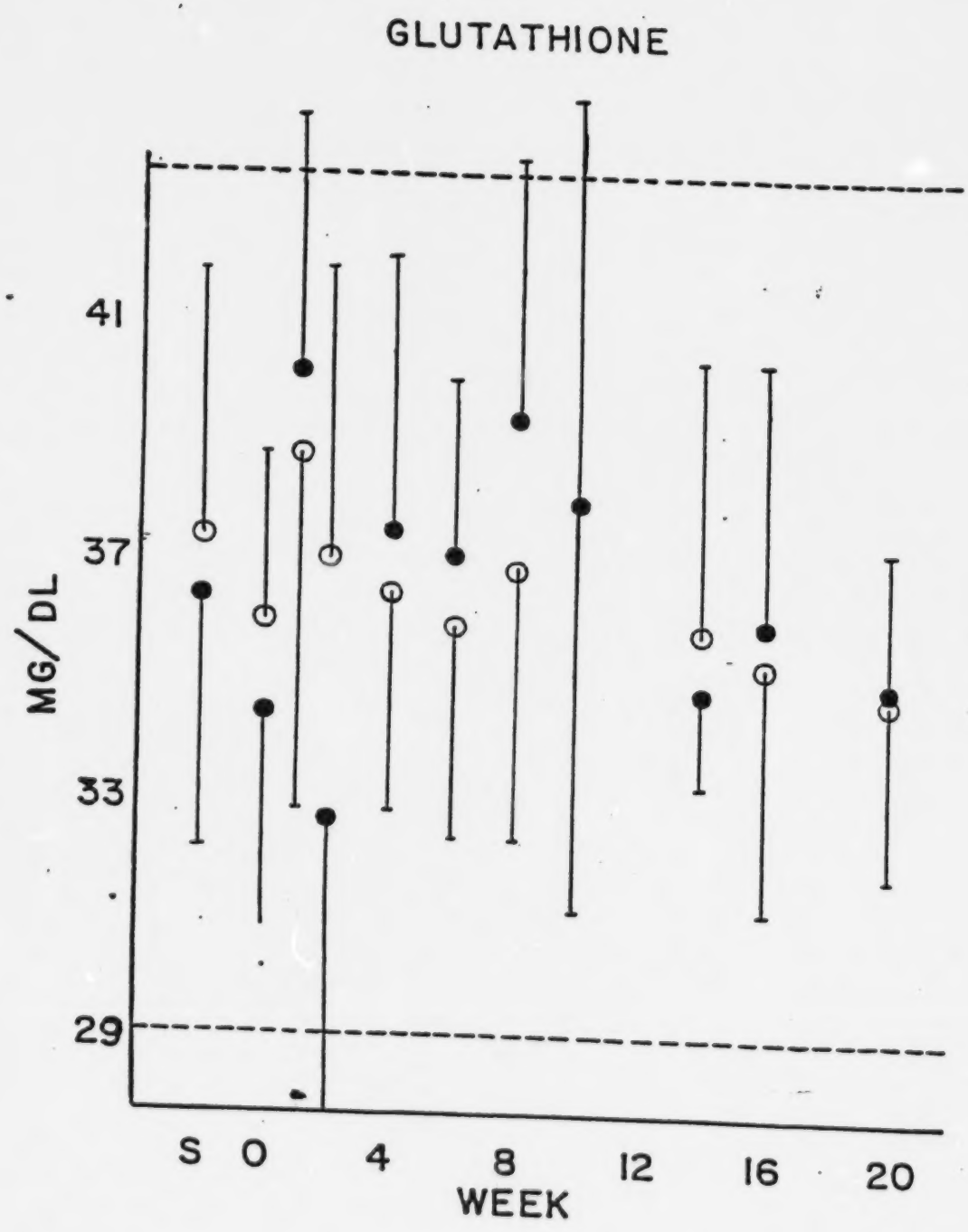


FIGURE 1c. GLUTATHIONE

Normal range ----
Control group ●
Chlorine group ○

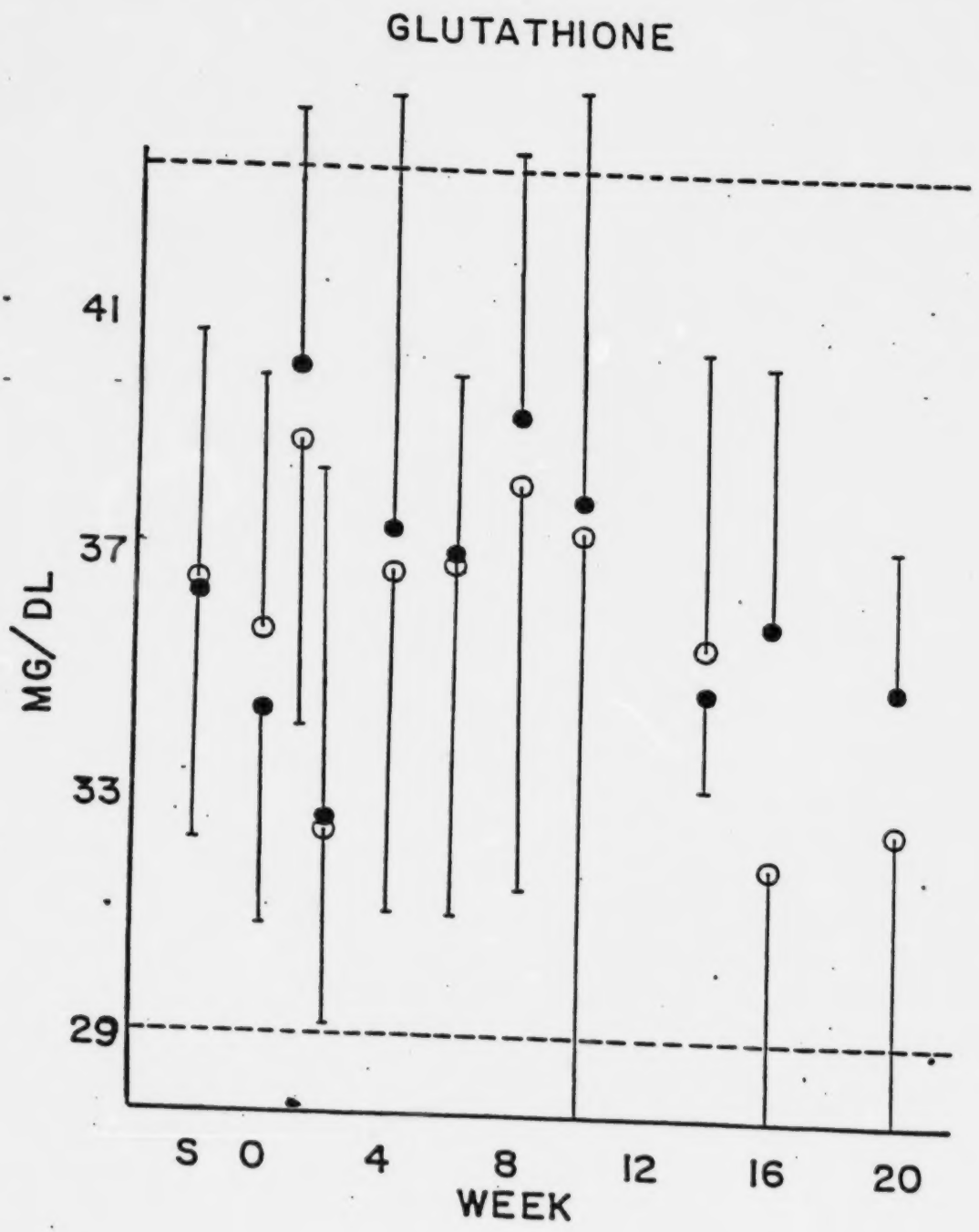
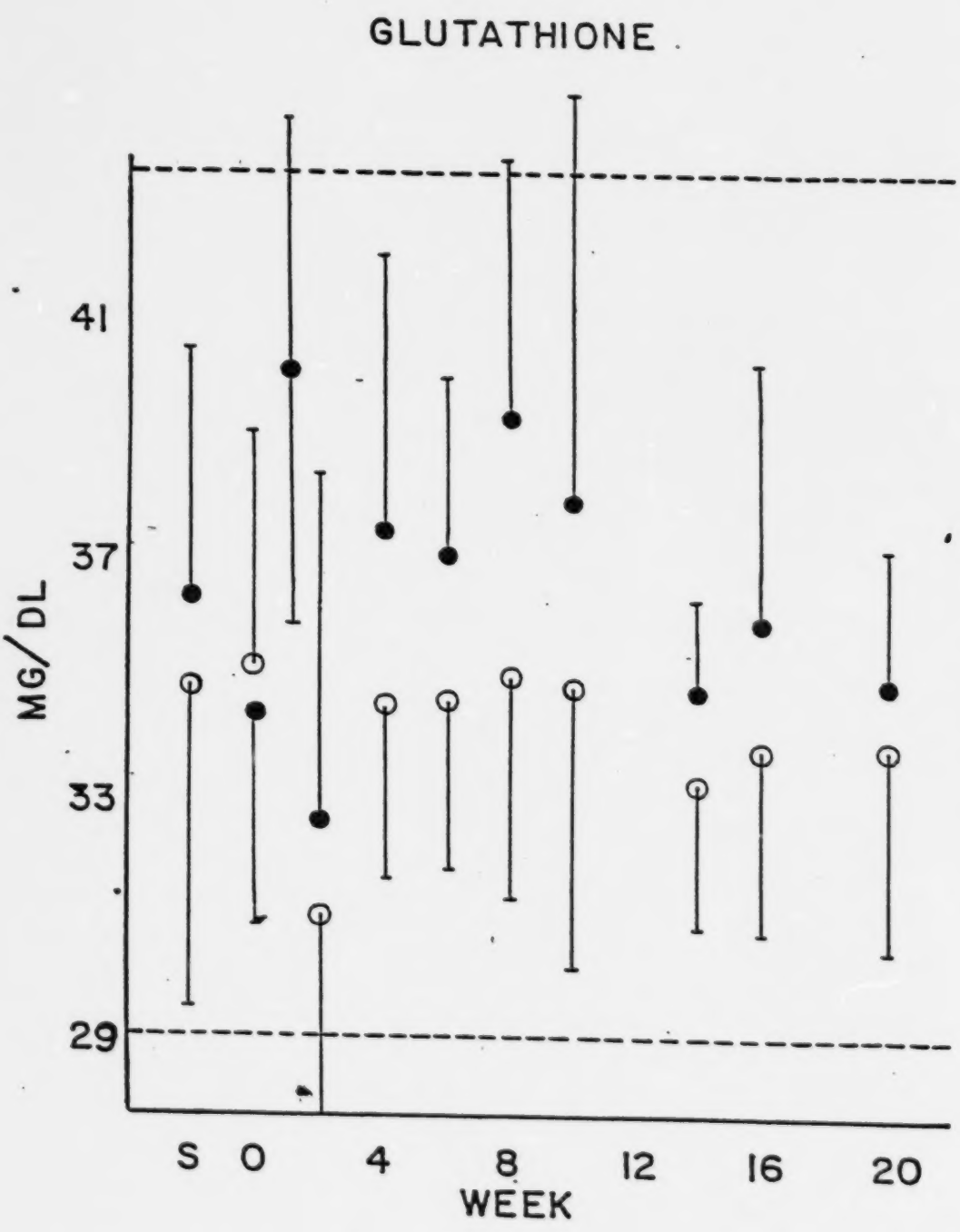


FIGURE 1d. GLUTATHIONE

Normal range ----
Control group ●
Chlorine dioxide group ○



49

FIGURE 1e. GLUTATHIONE

Normal range ----
Control group ●
Chloramine group ○

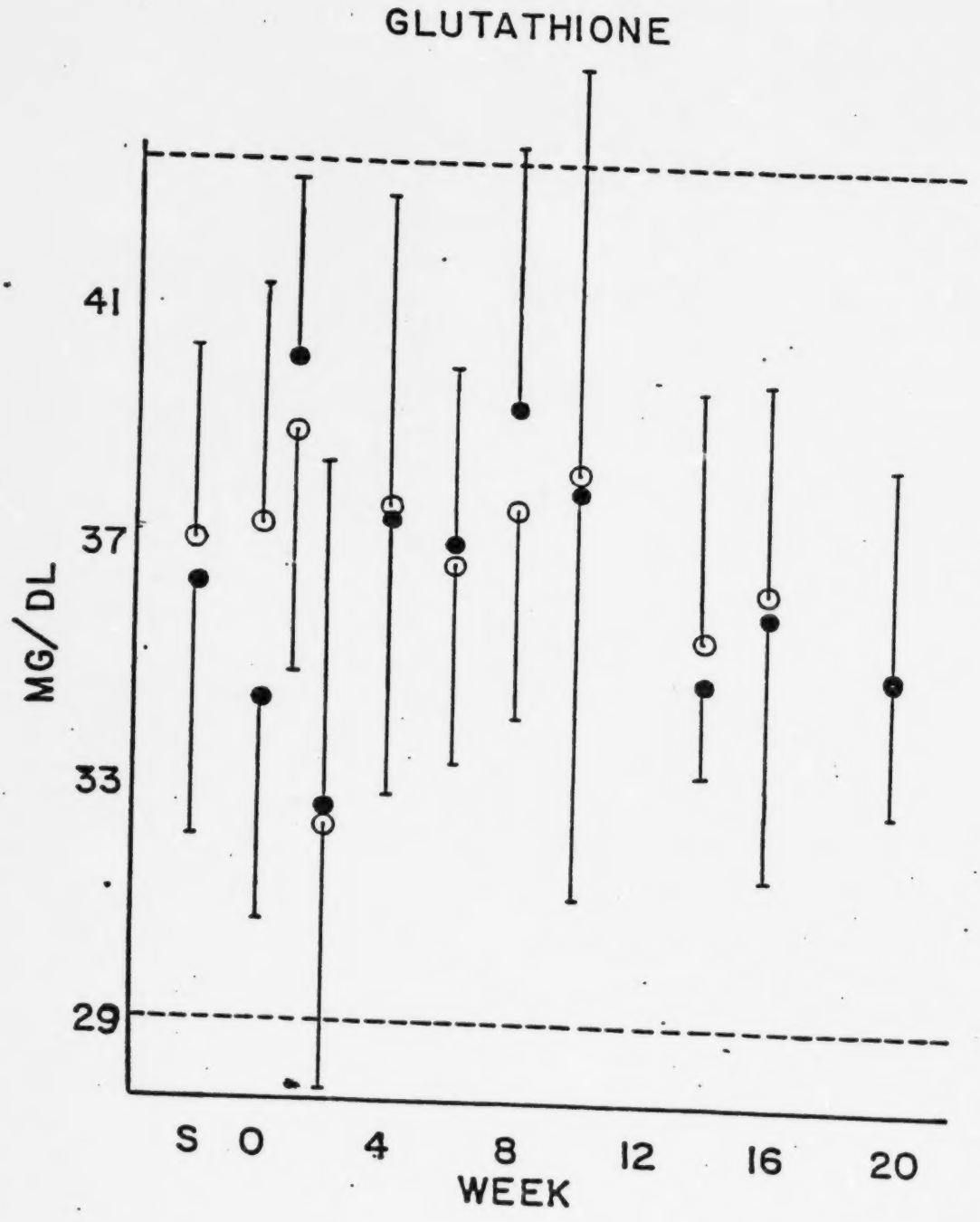


FIGURE 2a. HEMATOCRIT

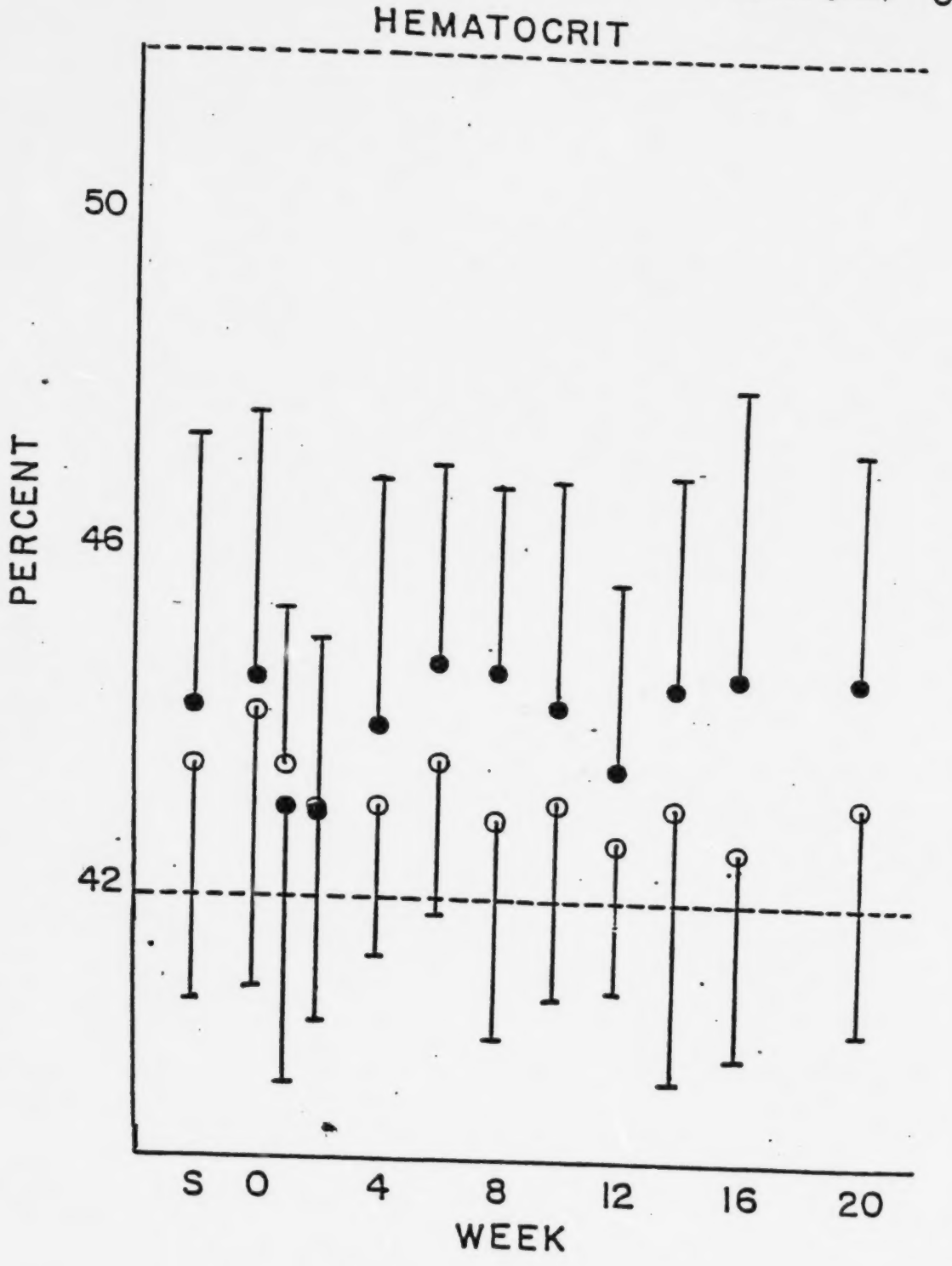


FIGURE 2b. HEMATOCRIT

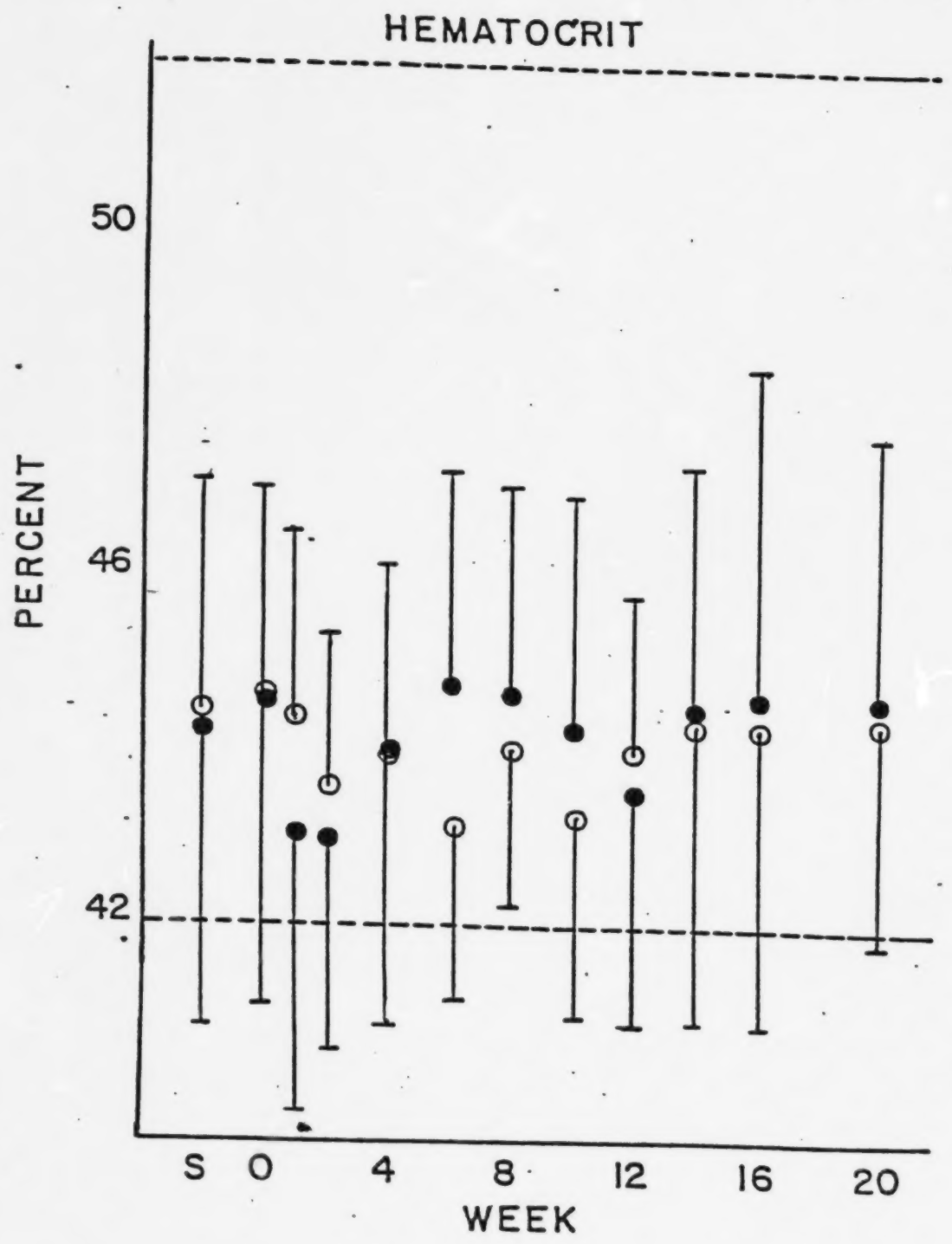


FIGURE 2c. HEMATOCRIT

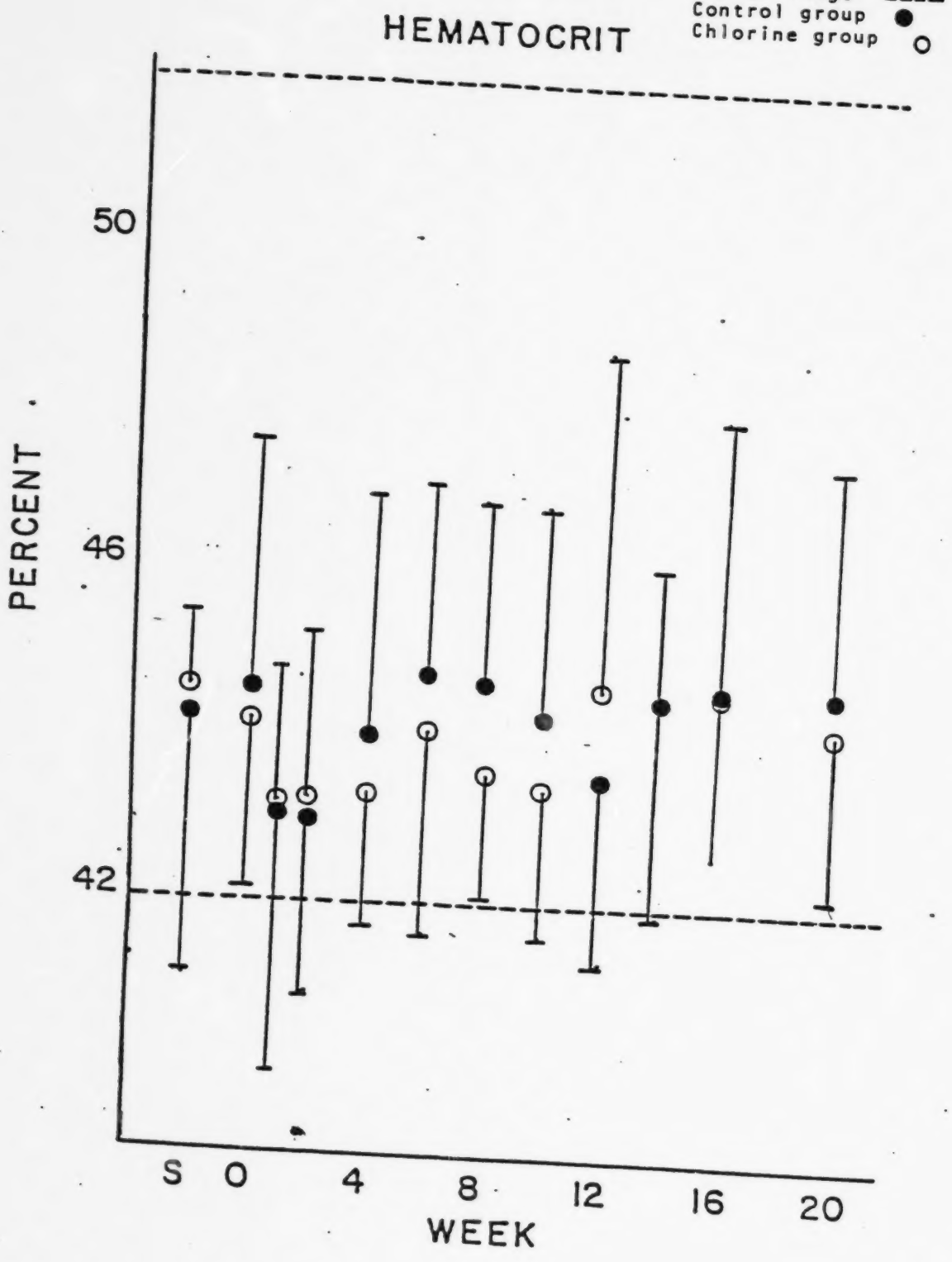


FIGURE 2d. HEMATOCRIT

Normal range ----
Control group ●
Chlorine dioxide group ○

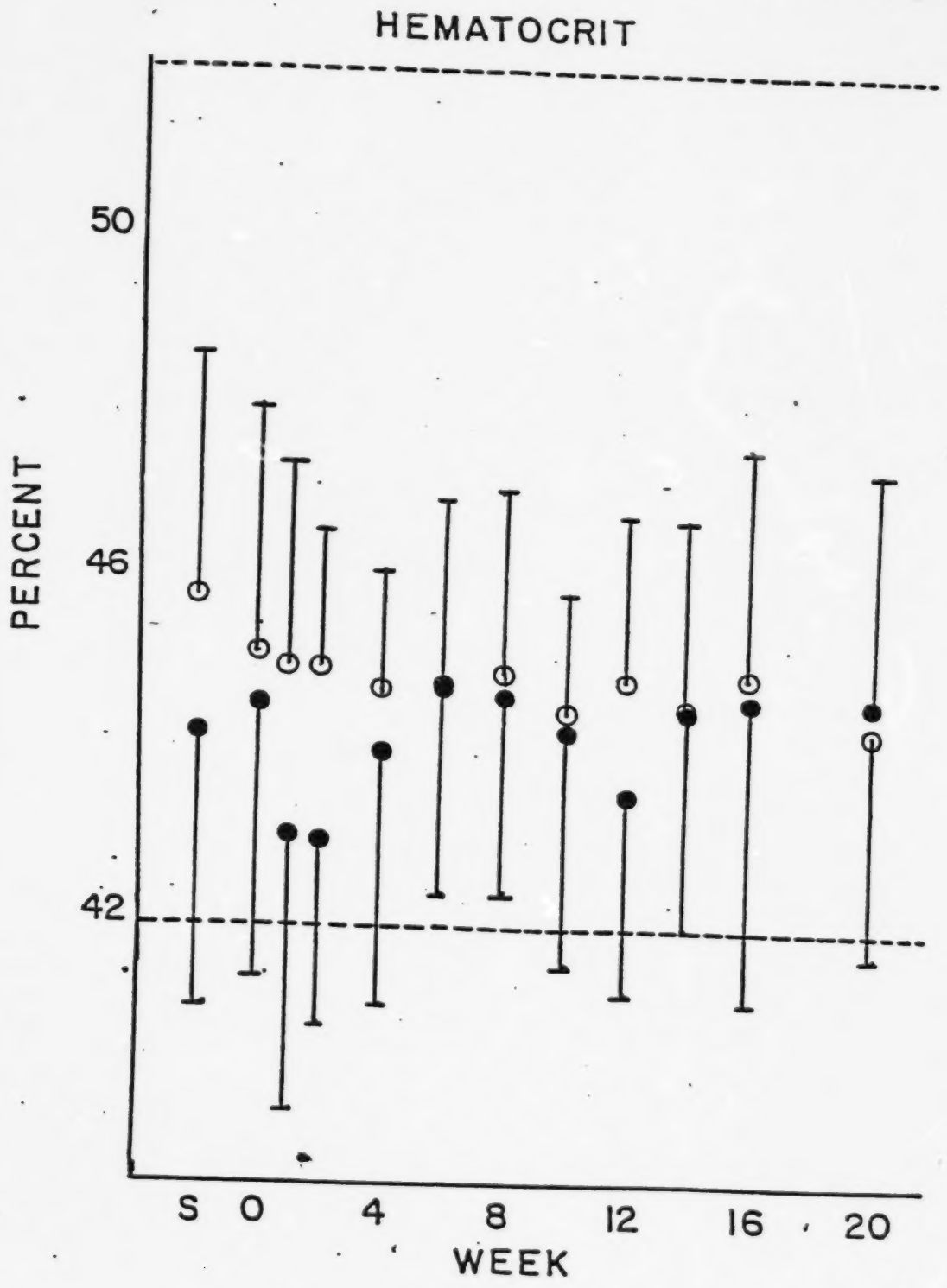


FIGURE 2e. HEMATOCRIT

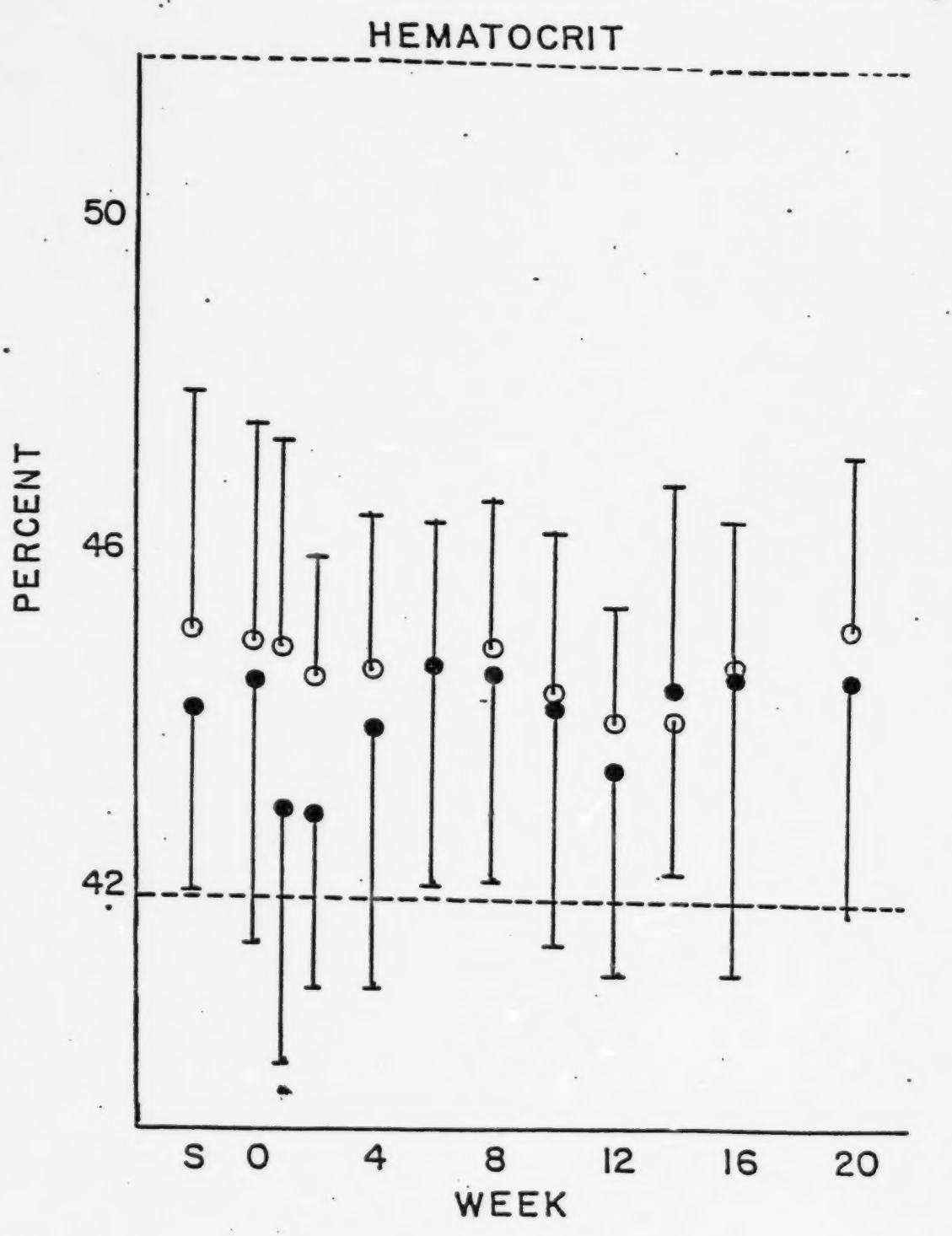


FIGURE 3a. RED BLOOD COUNT

Normal range ----

Control group ●

Chlorite group ○

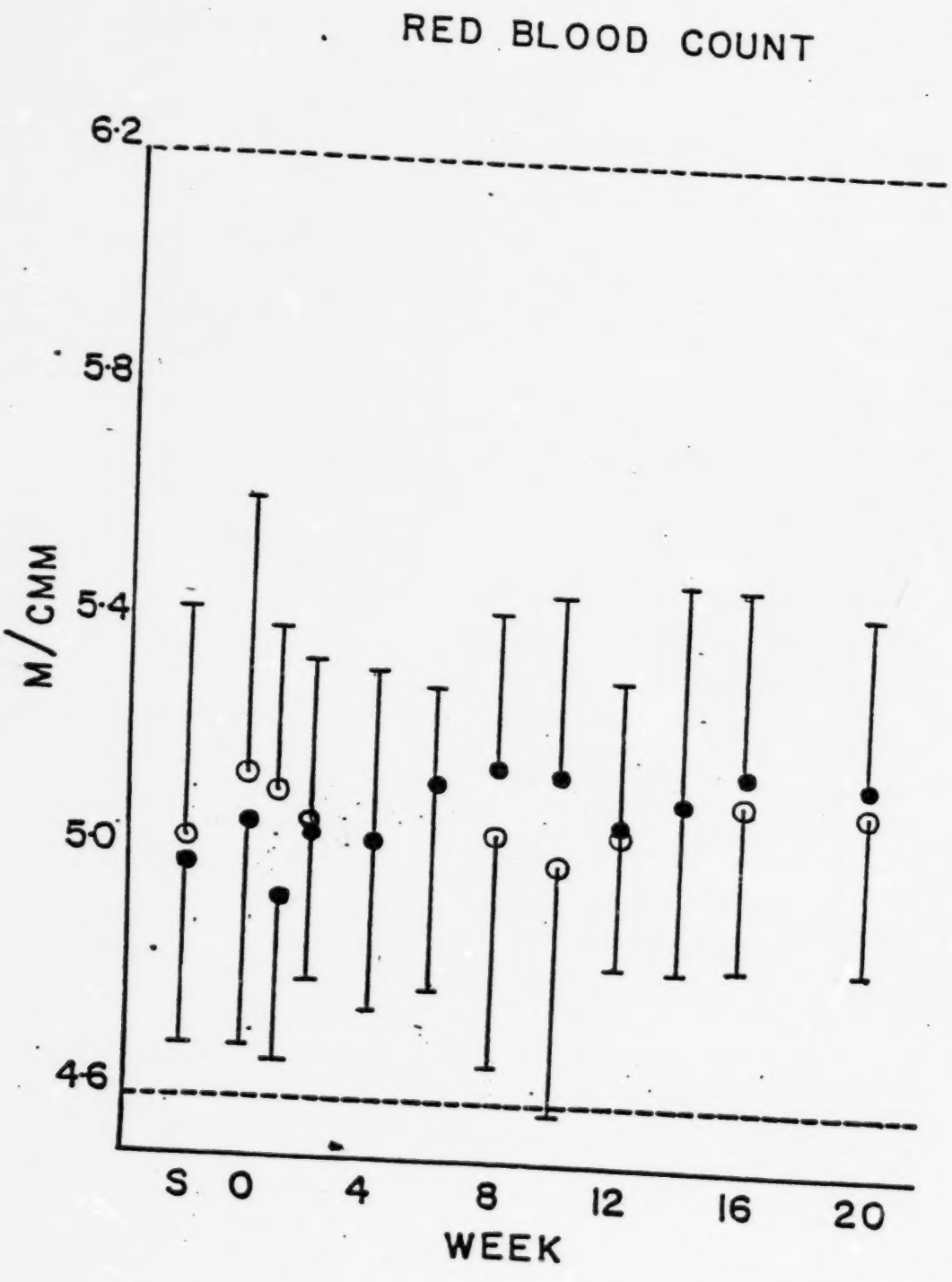


FIGURE 3b. RED BLOOD COUNT

Normal range ----

Control group ●

Chlorate group ○

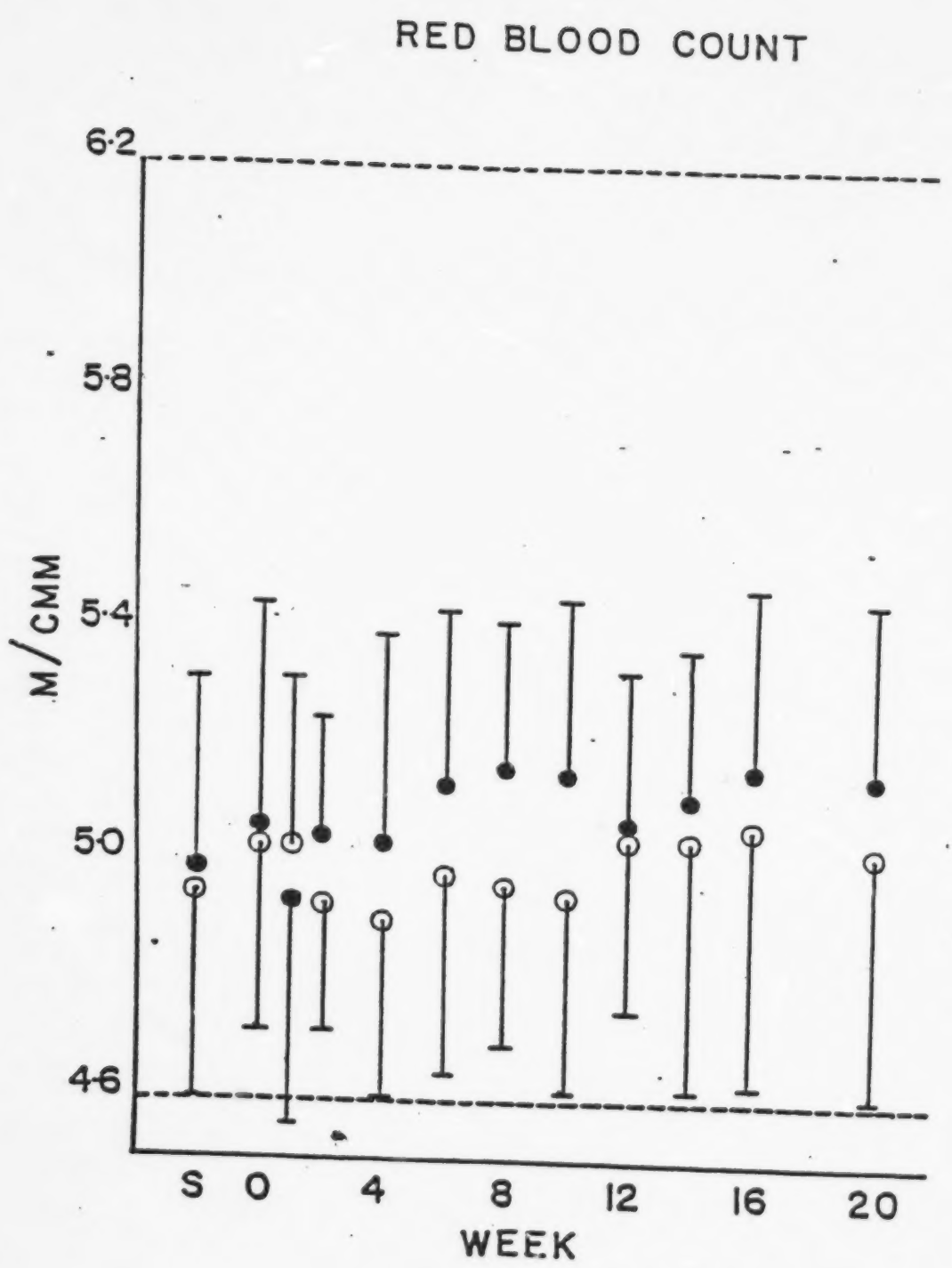


FIGURE 3c. RED BLOOD COUNT

Normal range ----

Control group ●

Chlorine group ○

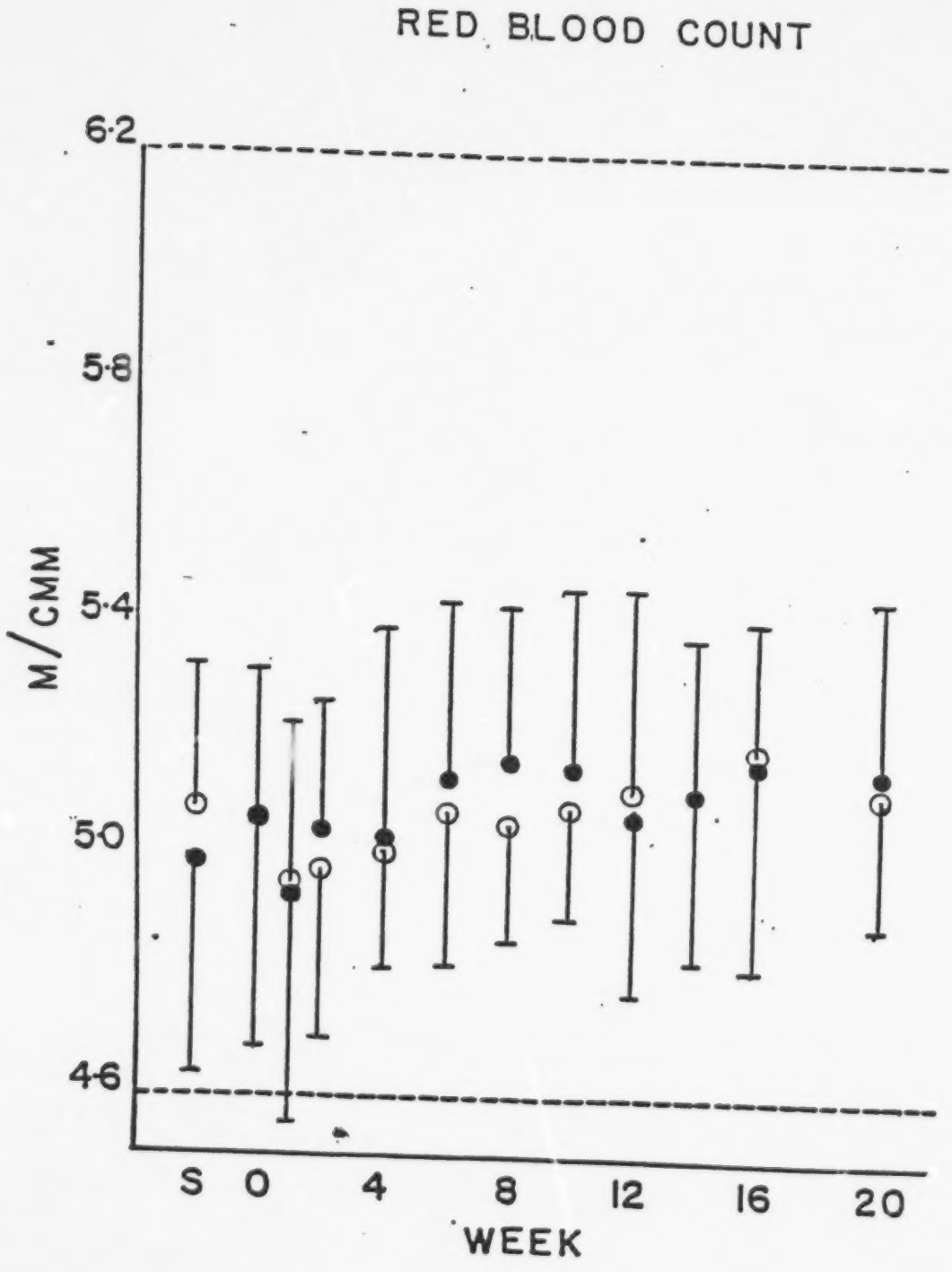


FIGURE 3d. RED BLOOD COUNT
Normal range ----
Control group ●
Chlorine dioxide group ○

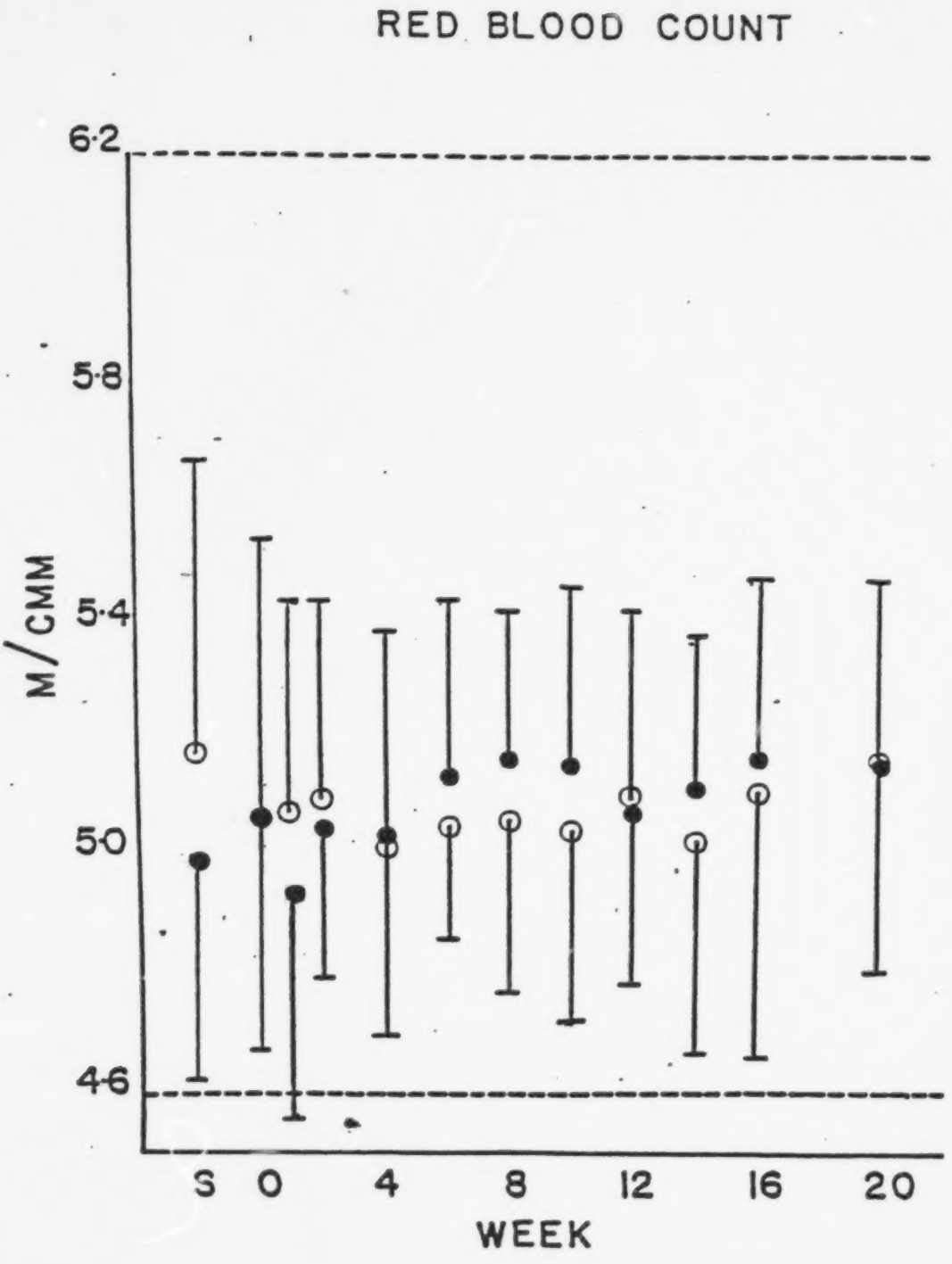


FIGURE 3e. RED BLOOD COUNT

Normal range ----

Control group ●

Chloramine group ○

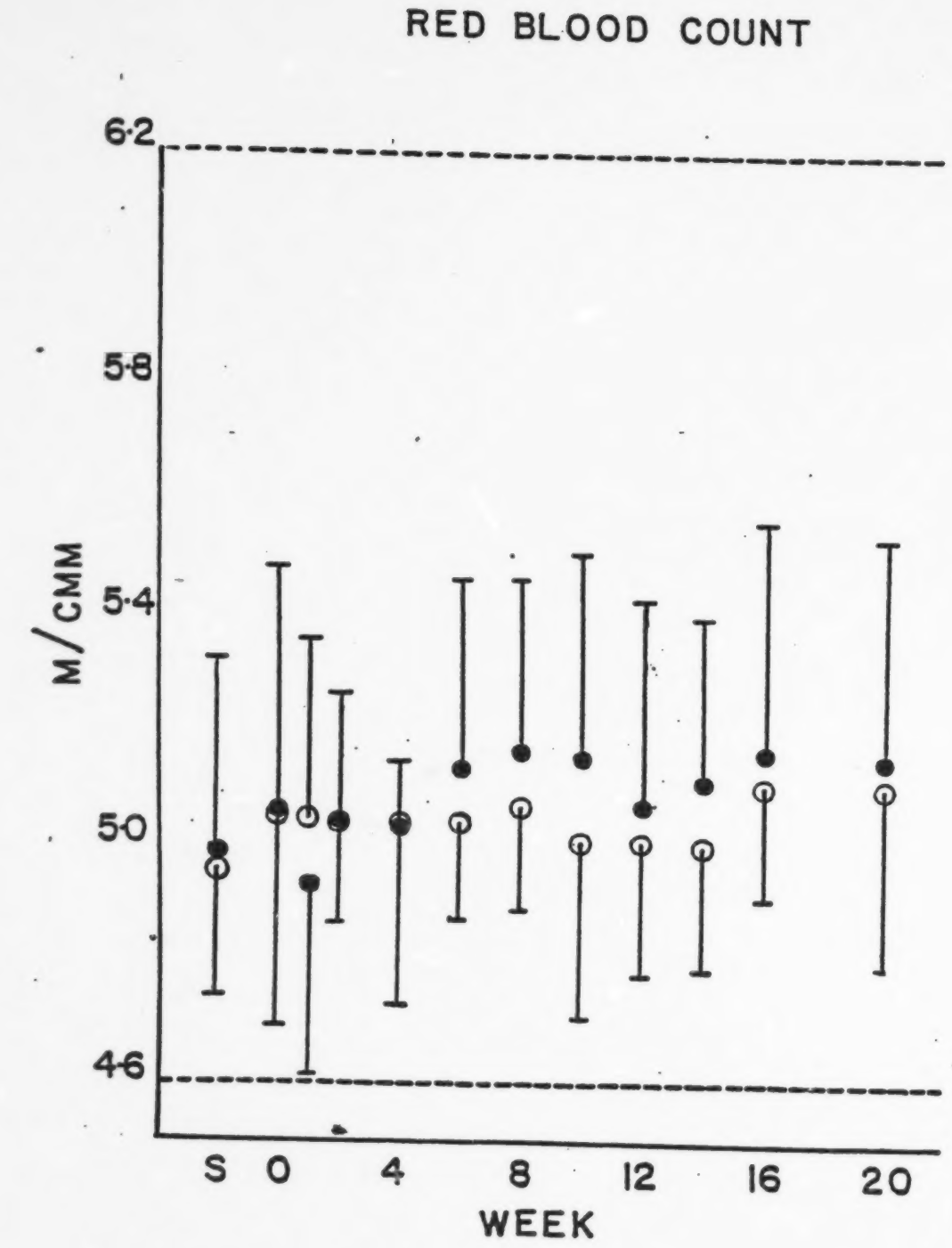


FIGURE 4a. HEMOGLOBIN

Normal range ----
Control group ●
Chlorite group ○

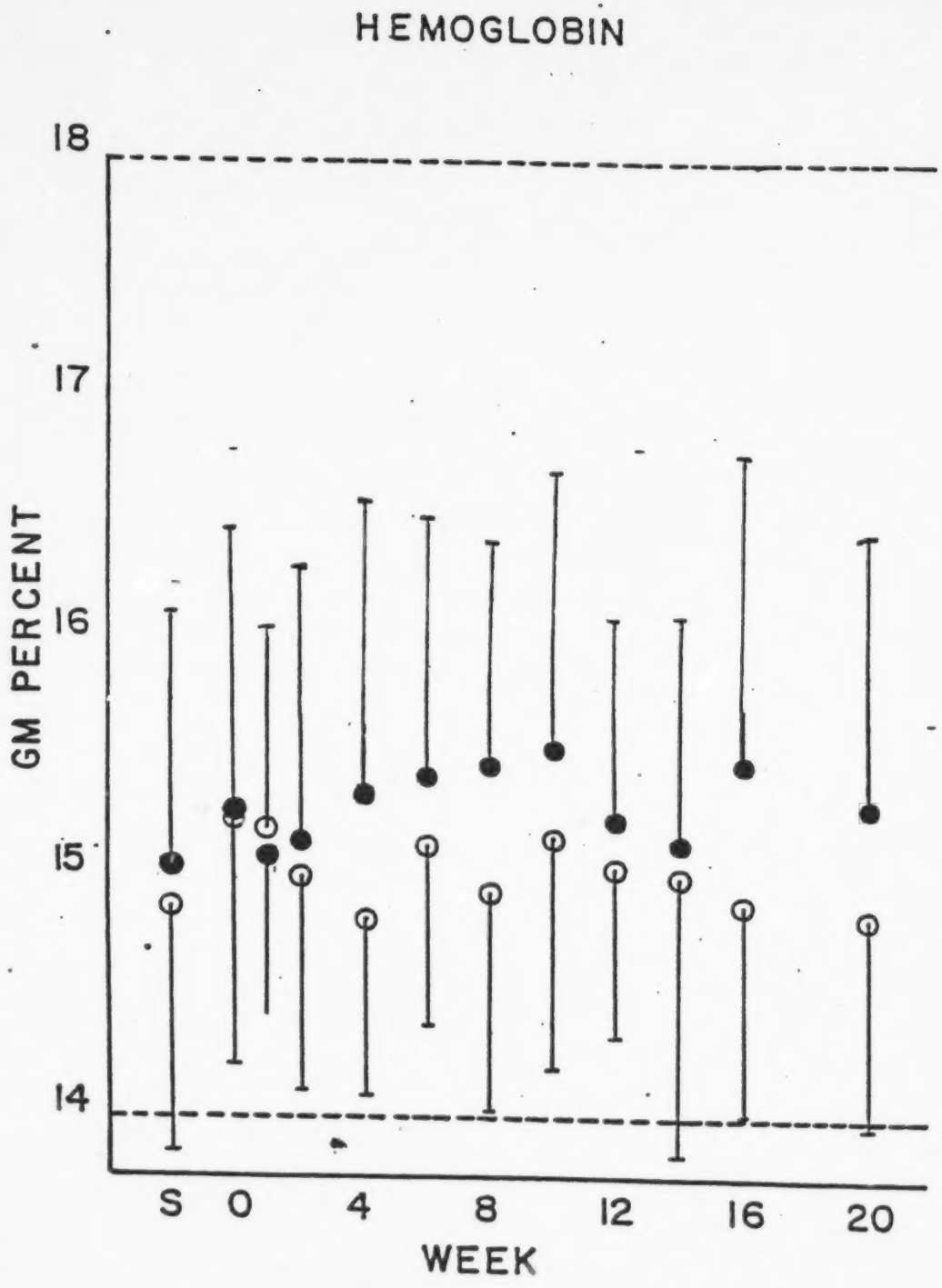


FIGURE 4b. HEMOGLOBIN

Normal range ----
Control group ●
Chlorate group ○

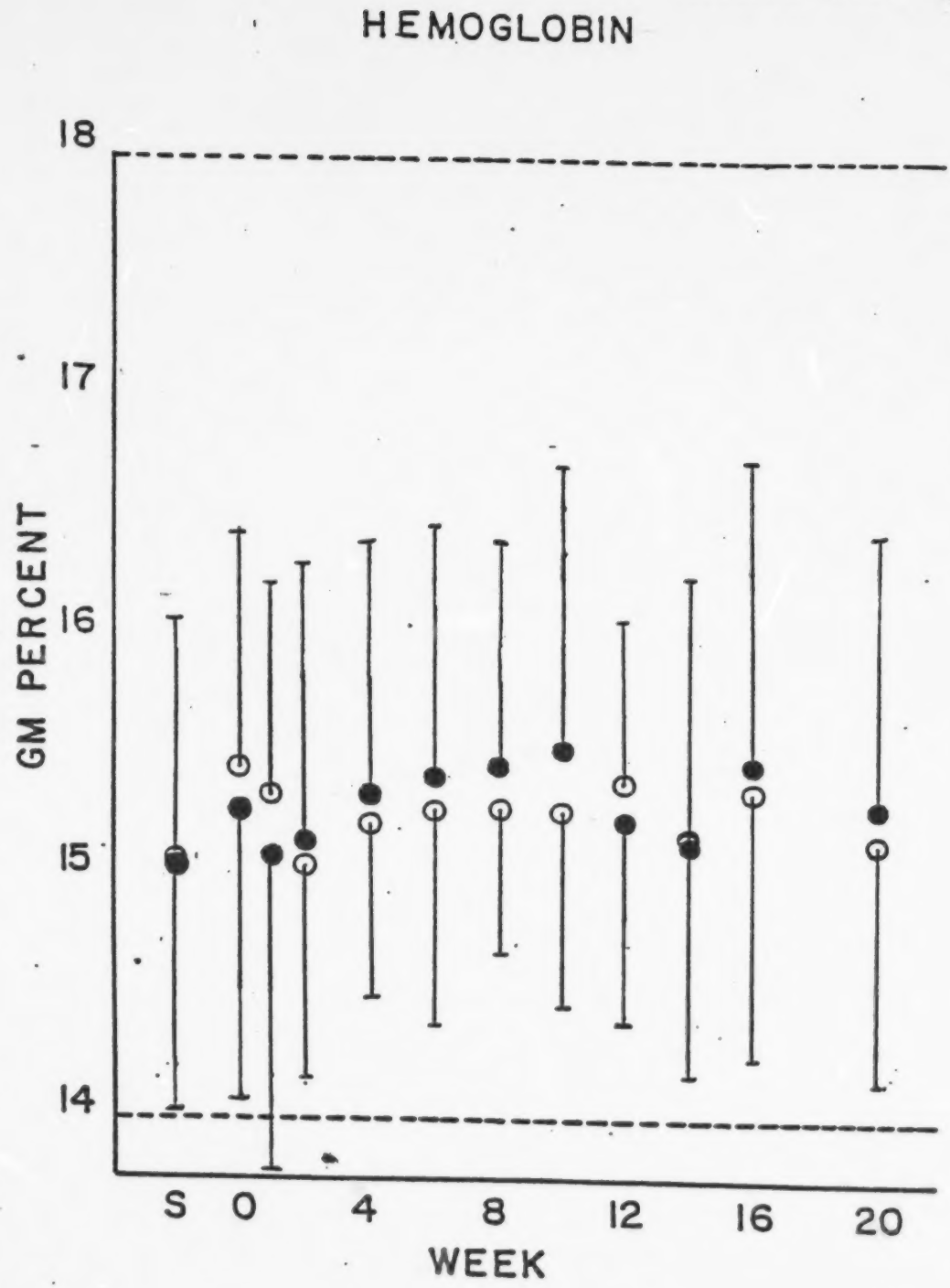


FIGURE 4c. HEMOGLOBIN

Normal range ----
Control group ●
Chlorine group ○

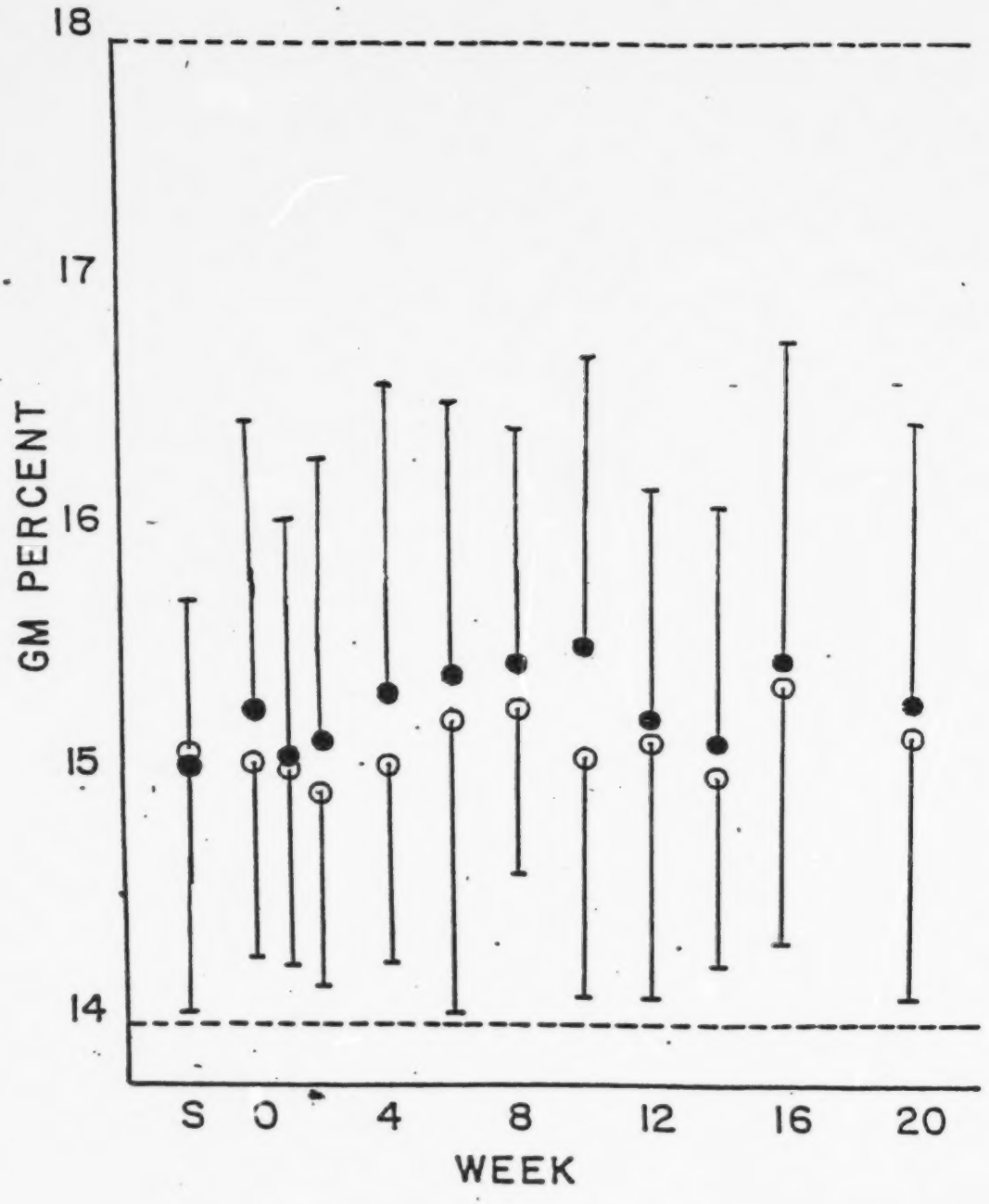


FIGURE 4d. HEMOGLOBIN

Normal range ----
Control group ●
Chlorine dioxide group ○

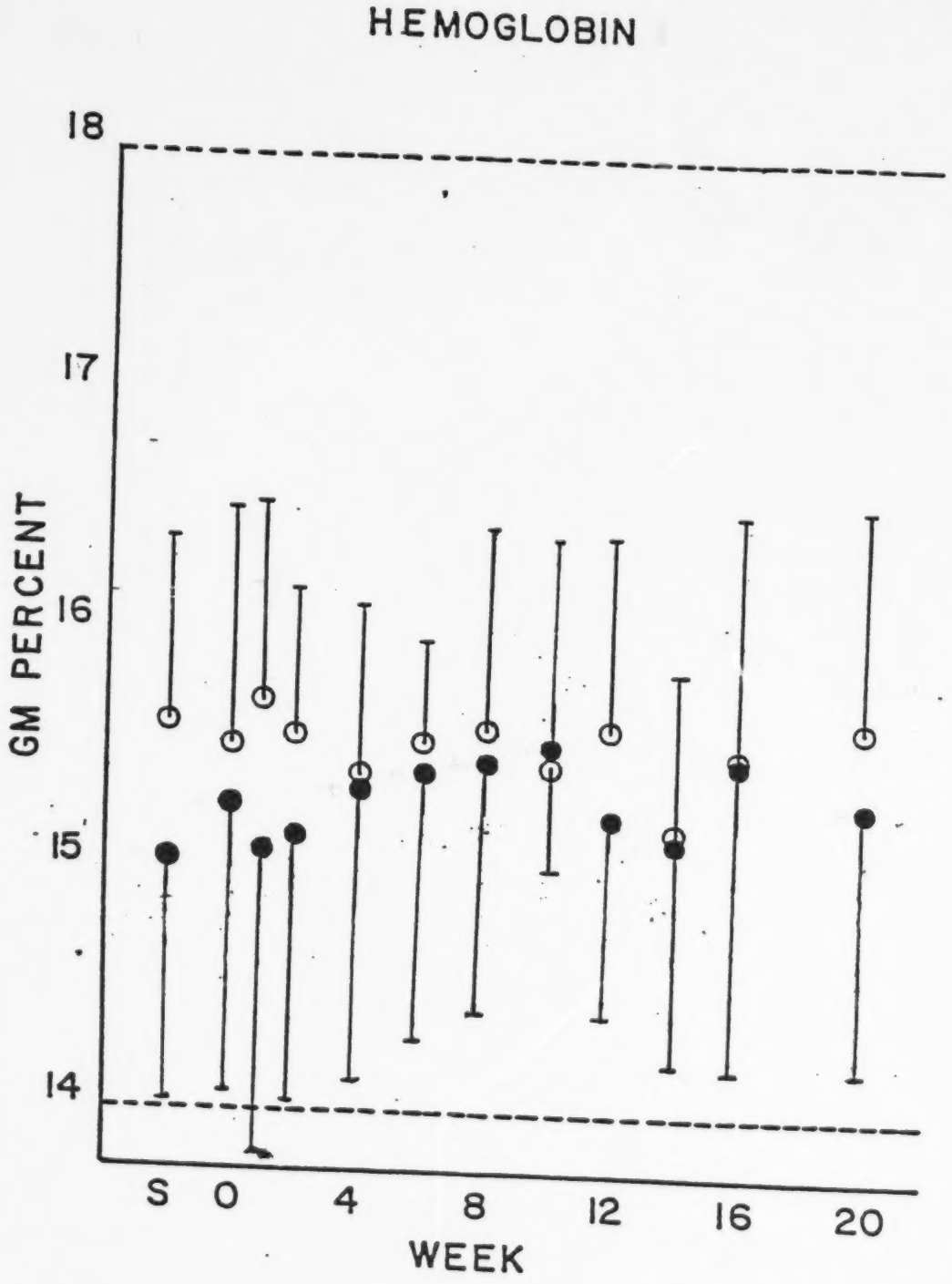


FIGURE 4e. HEMOGLOBIN

Normal range ----
Control group ●
Chloramine group ○

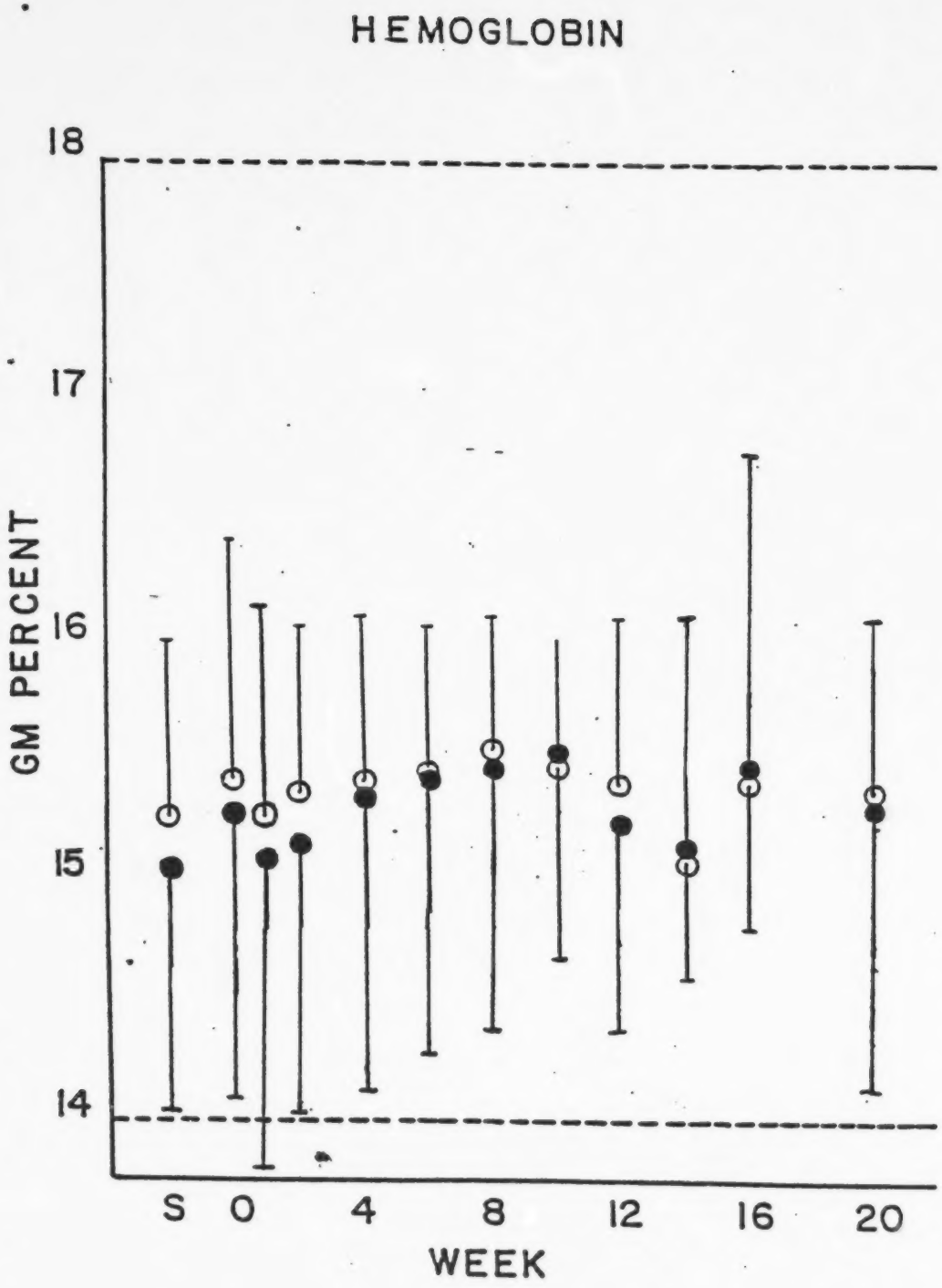


FIGURE 5a. T-3 UPTAKE

Normal range ----
Control group ●
Chlorite group ○

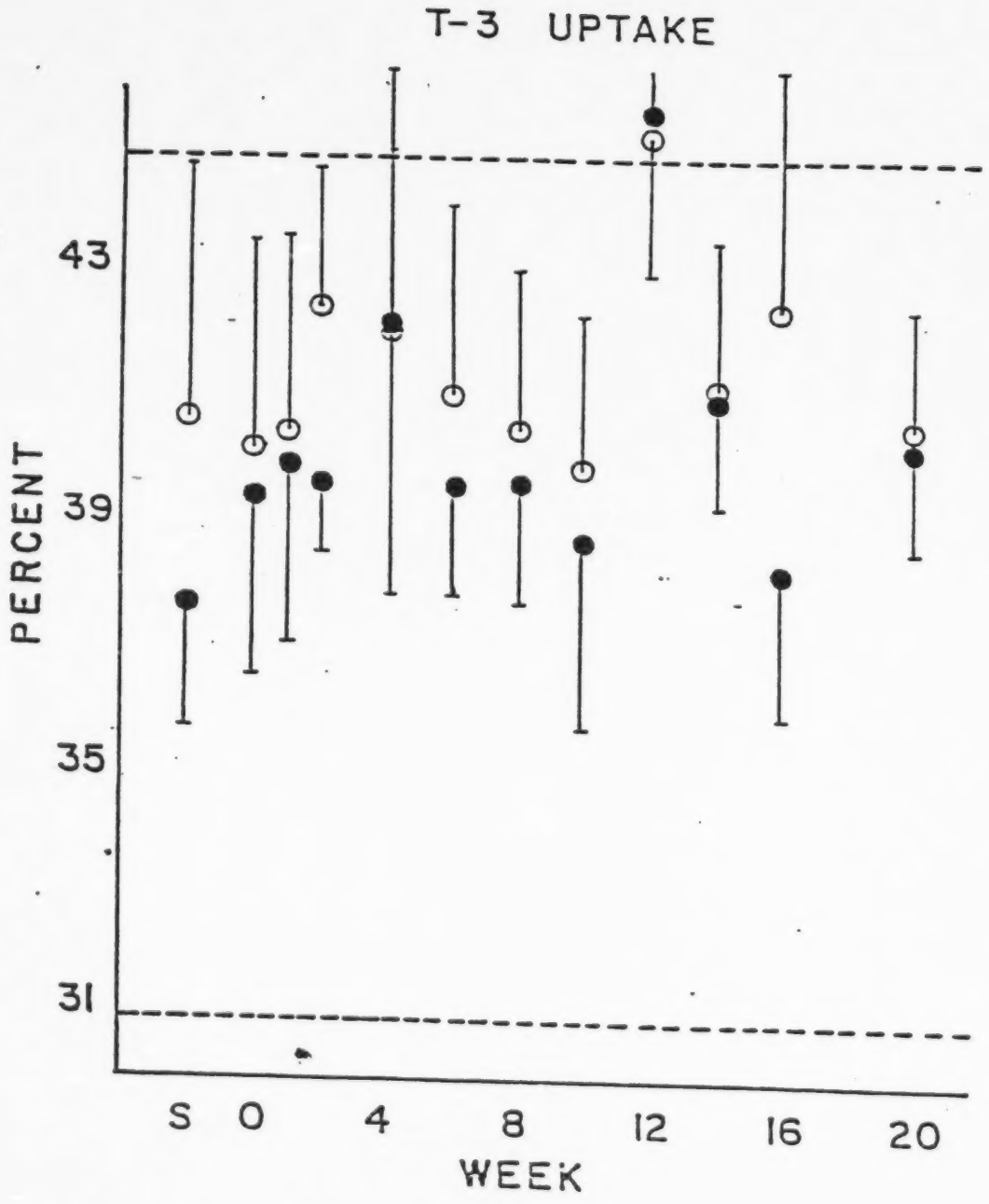


FIGURE 5b. T-3 UPTAKE

Normal range ----
Control group ●
Chlorate group ○

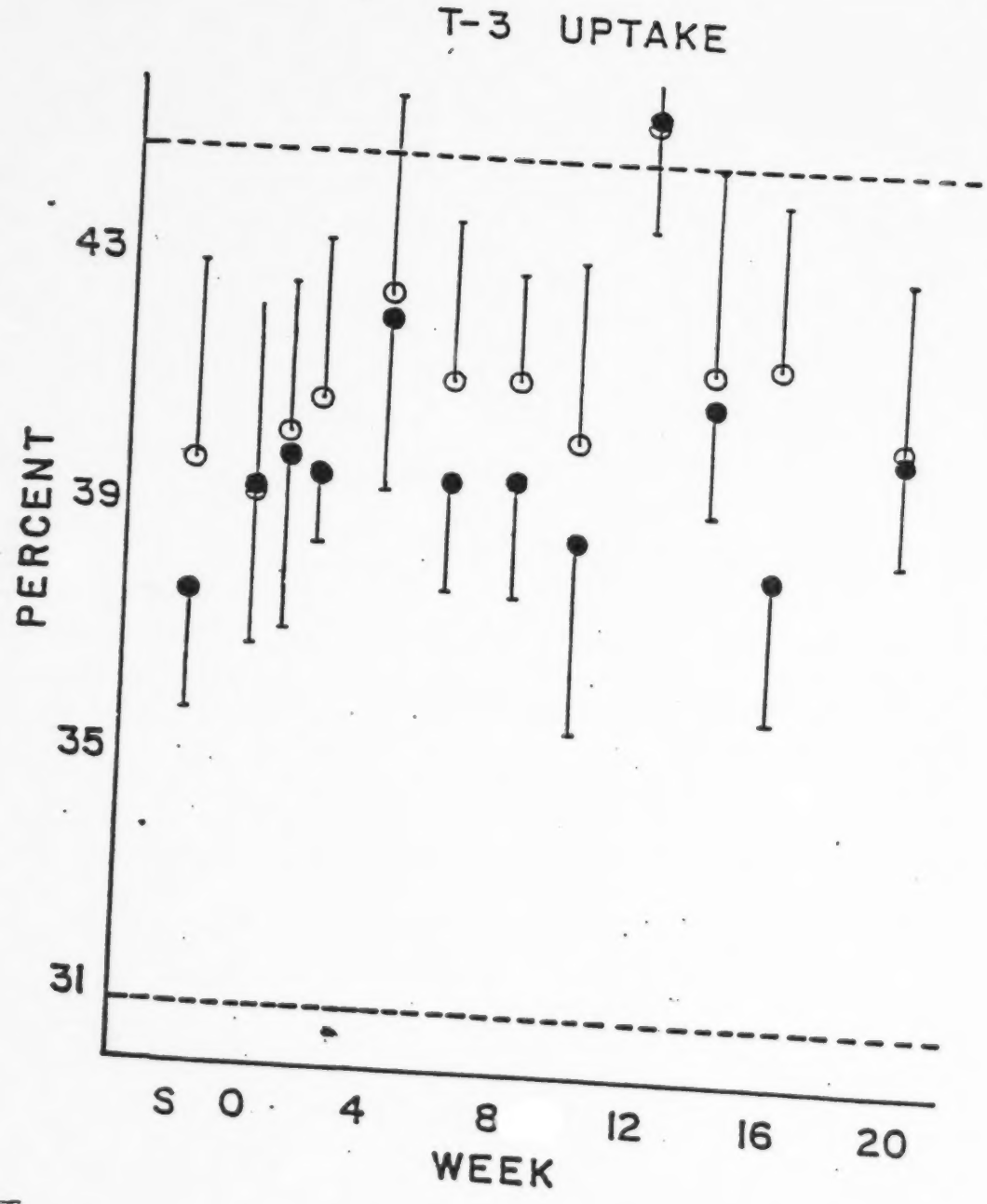


FIGURE 5c. T-3 UPTAKE

Normal range ----
Control group ●
Chlorine group ○

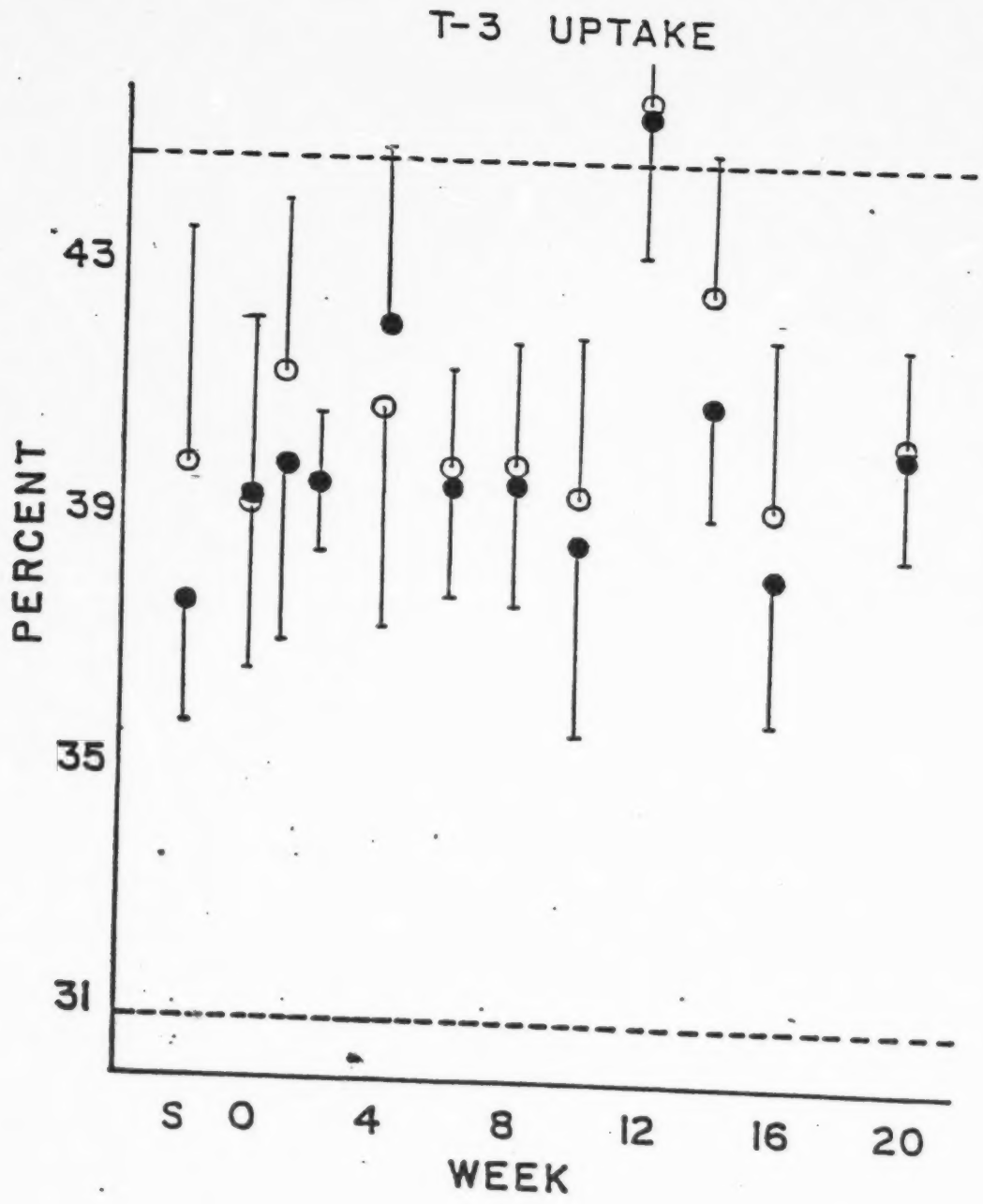


FIGURE 5d. T-3 UPTAKE

Normal range ----
Control group ●
Chlorine dioxide group ○

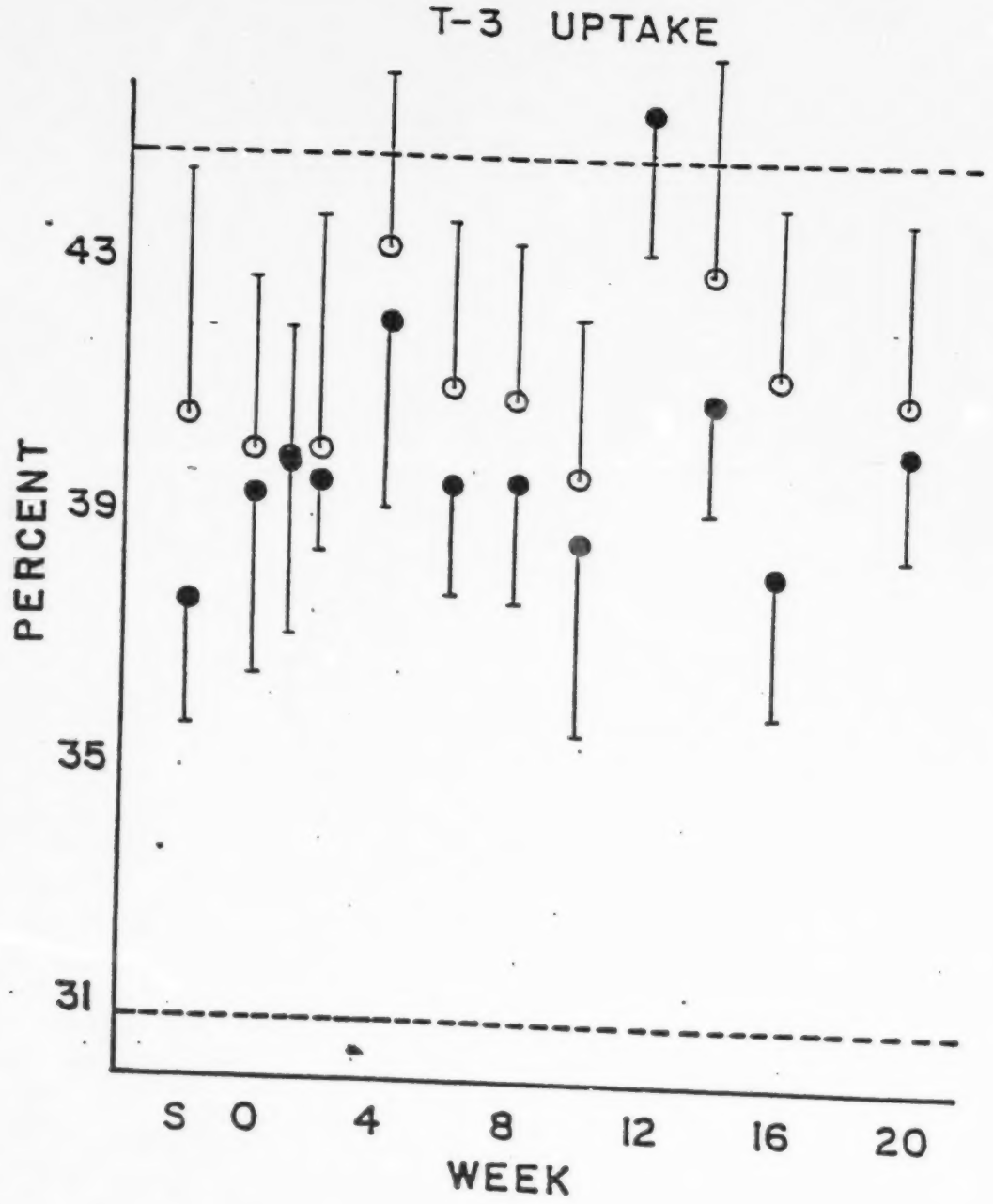


FIGURE 5e. T-3 UPTAKE

Normal range ----
Control group ●
Chloramine group O

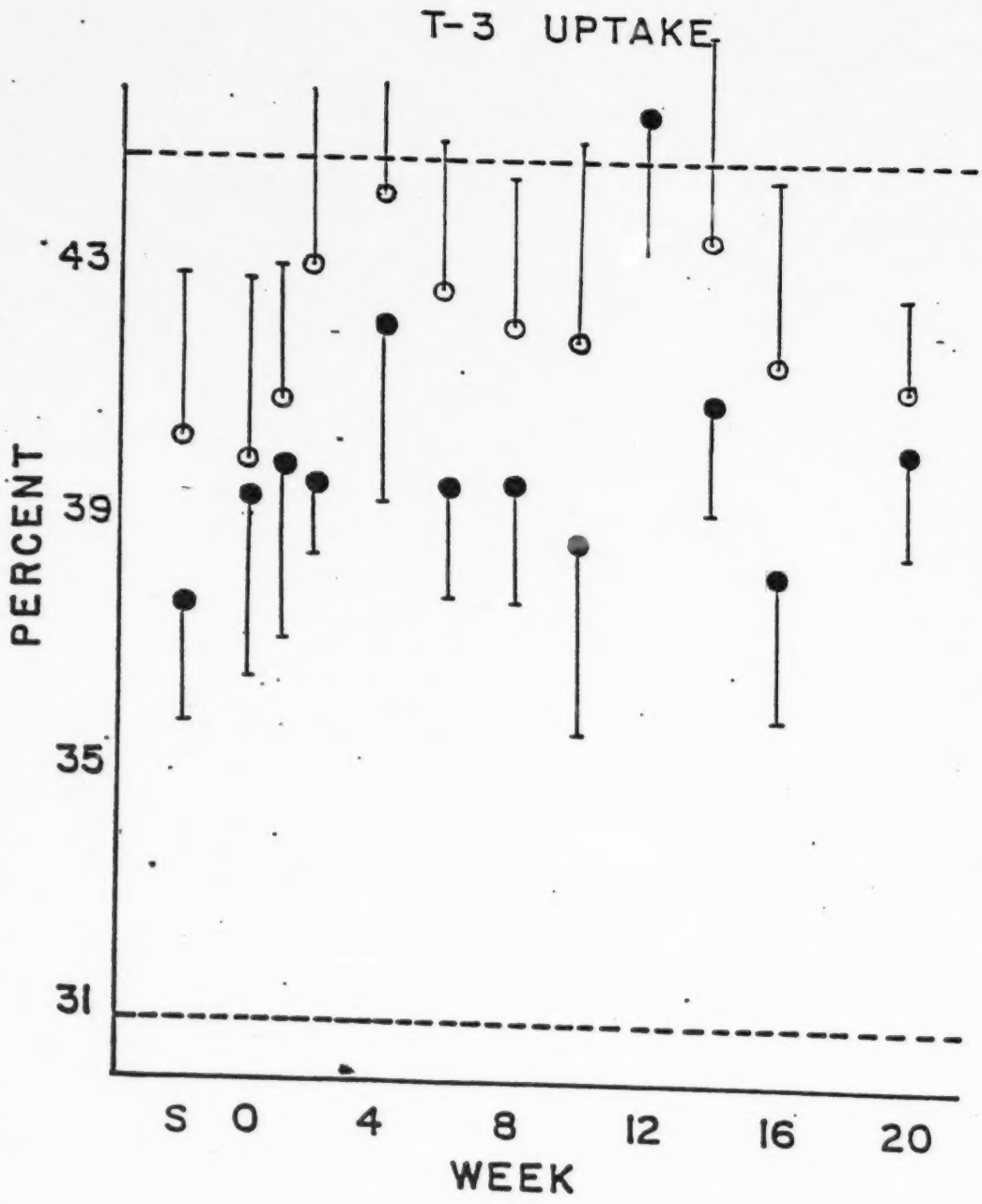


FIGURE 6a. UREA NITROGEN

Normal range ----
Control group ●
Chlorite group ○

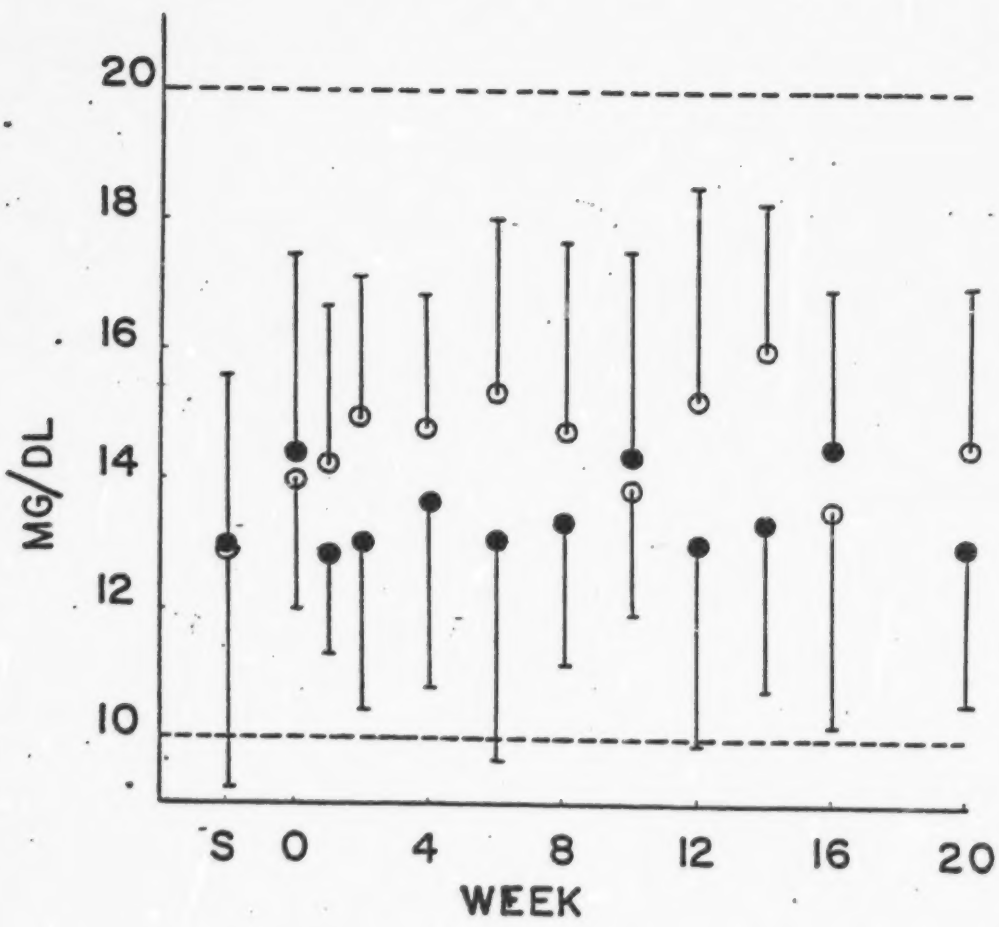


FIGURE 6b. UREA NITROGEN

Normal range ----
Control group ●
Chlorate group ○

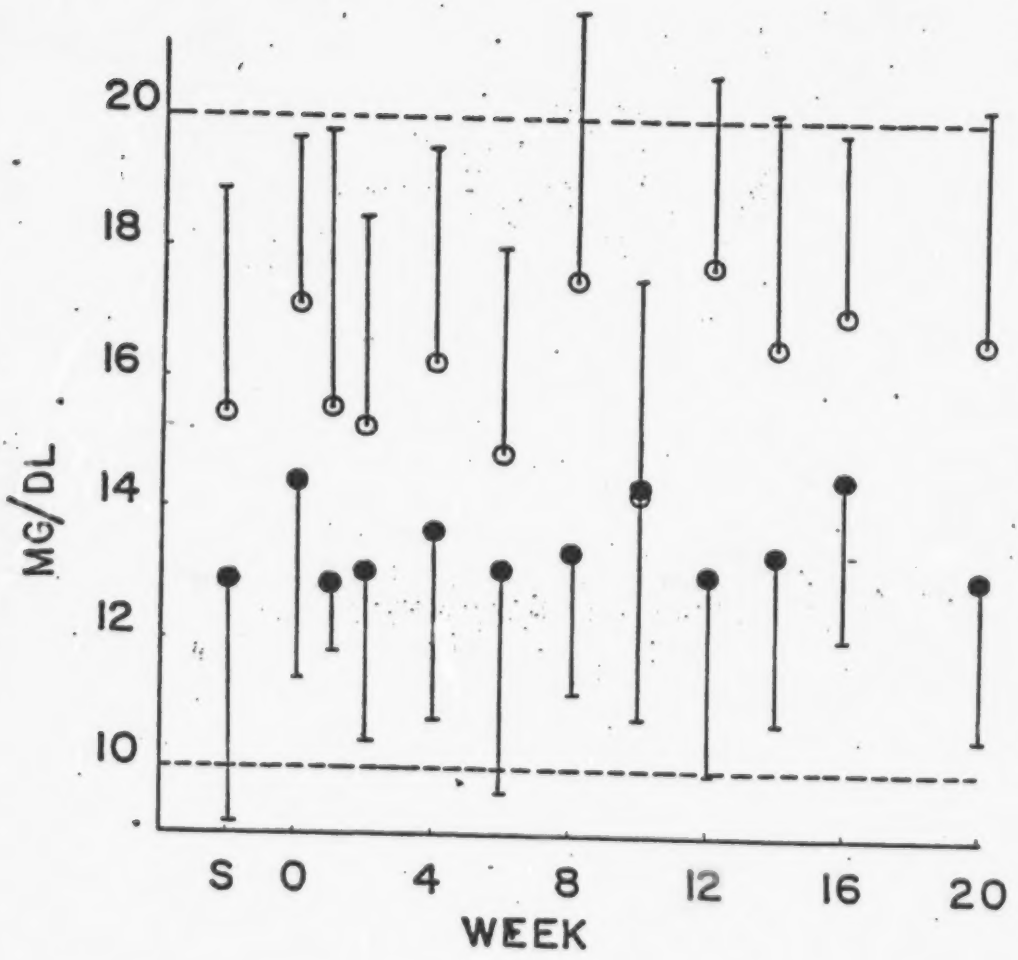


FIGURE 6c. UREA NITROGEN

Normal range ----
Control group ●
Chlorine group ○

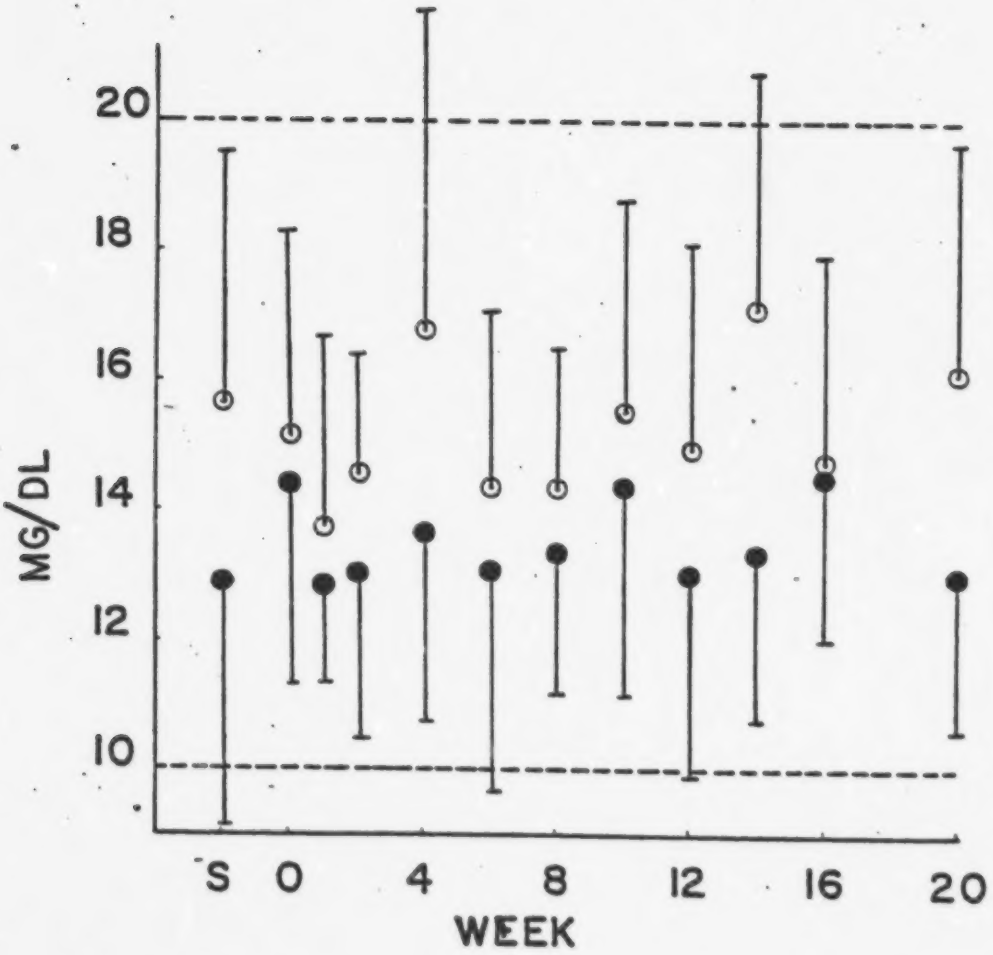


FIGURE 6d. UREA NITROGEN

Normal range ----
Control group ●
Chlorine dioxide group O

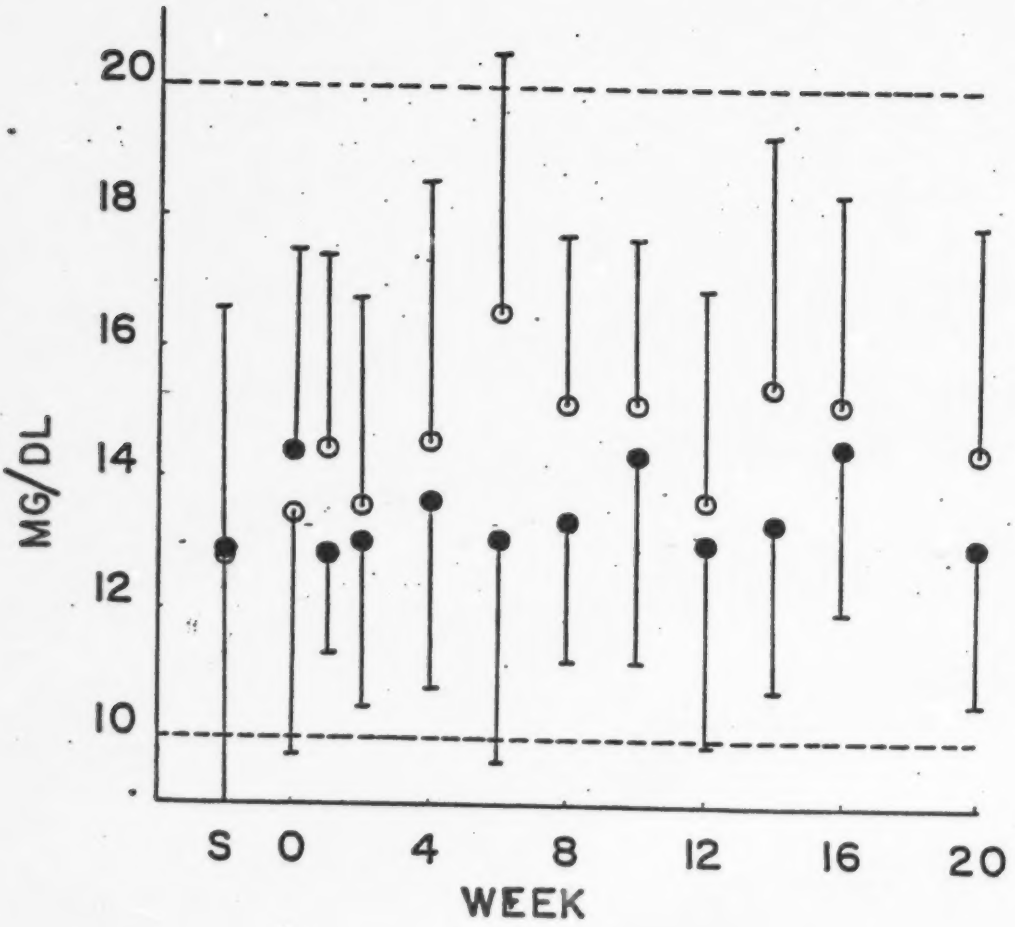


FIGURE 6e. UREA NITROGEN

Normal range ----
Control group ●
Chloramine group O

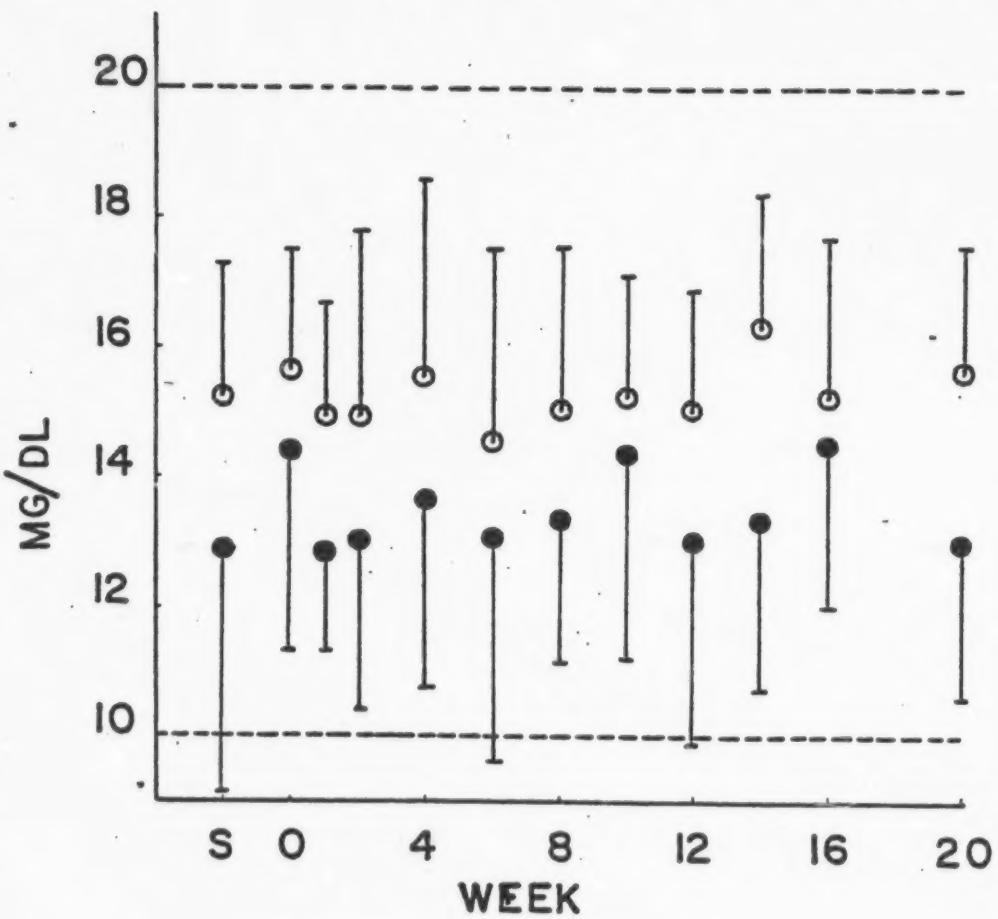


FIGURE 7a. MEAN CORPUSCULAR HEMOGLOBIN
Normal range ----
Control group ●
Chlorite group ○

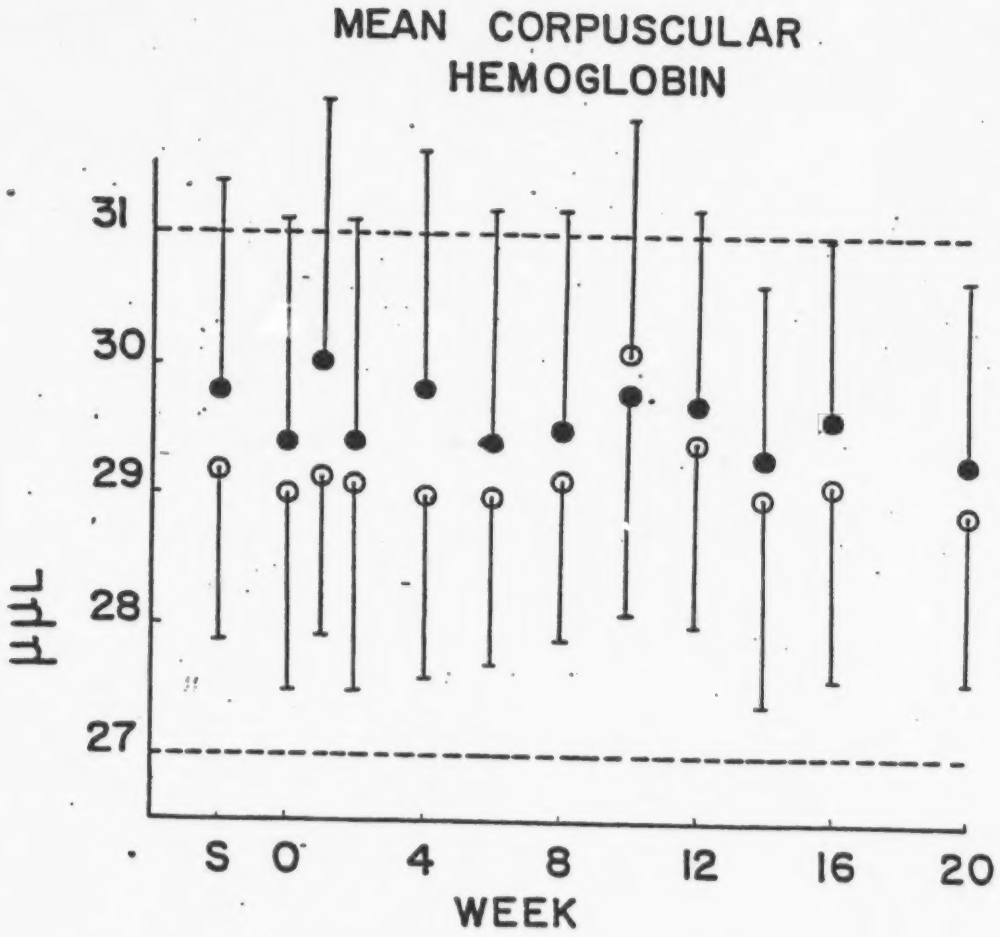


FIGURE 7b. MEAN CORPUSCULAR HEMOGLOBIN
Normal range ----
Control group ●
Chlorate group ○

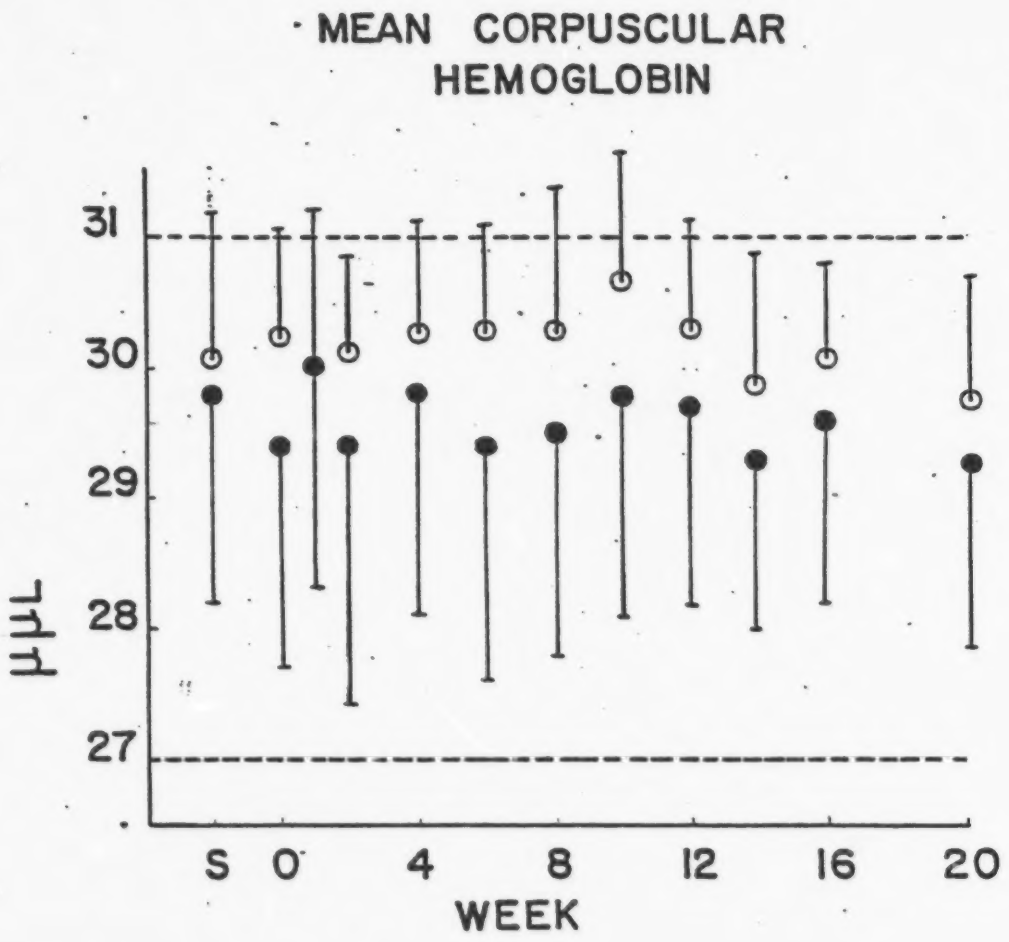


FIGURE 7c. MEAN CORPUSCULAR HEMOGLOBIN
Normal range ----
Control group ●
Chlorine group ○

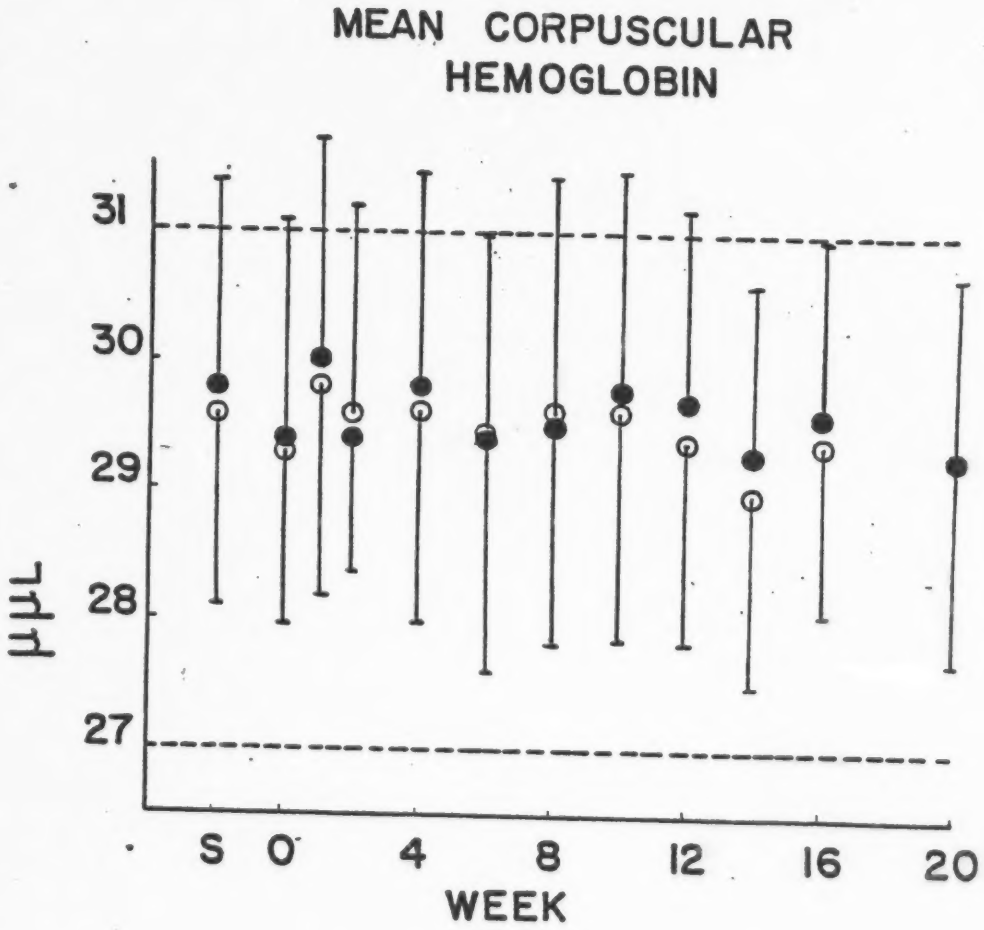


FIGURE 7d. MEAN CORPUSCULAR HEMOGLOBIN
Normal range ----
Control group ●
Chlorine dioxide group ○

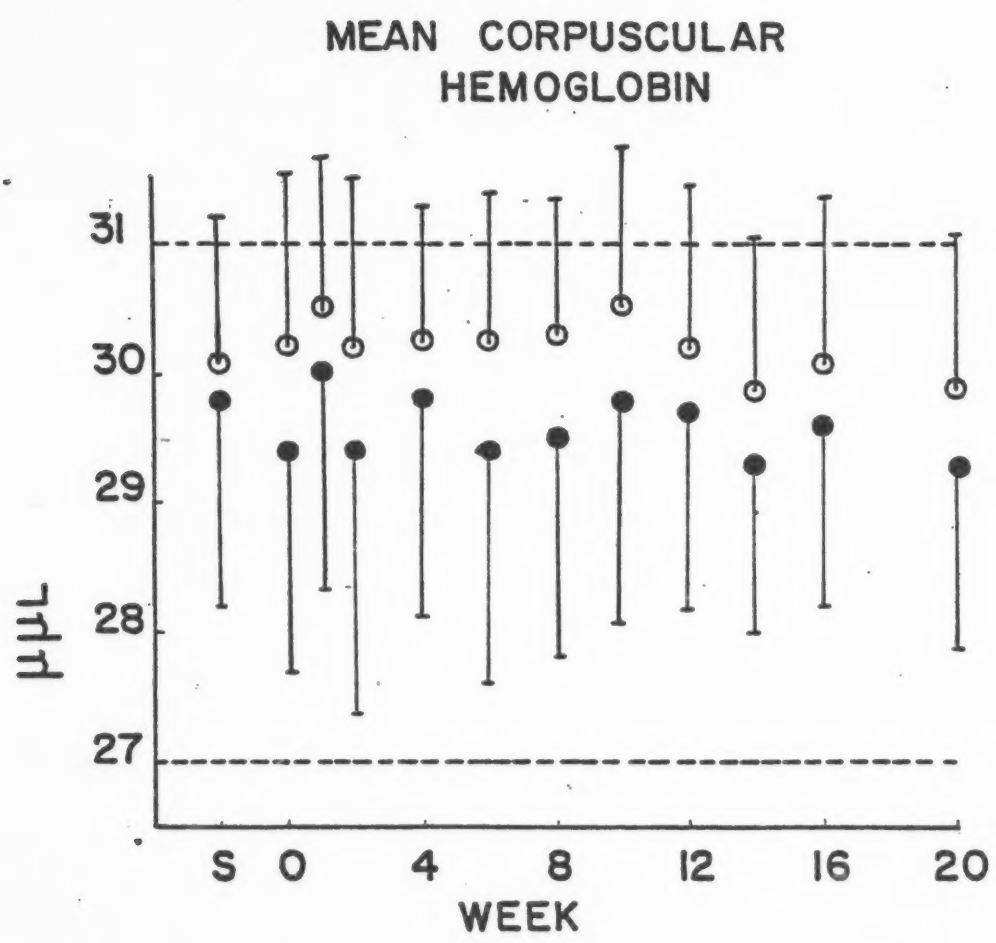


FIGURE 7e. MEAN CORPUSCULAR HEMOGLOBIN
Normal range ----
Control group ●
Chloramine group ○

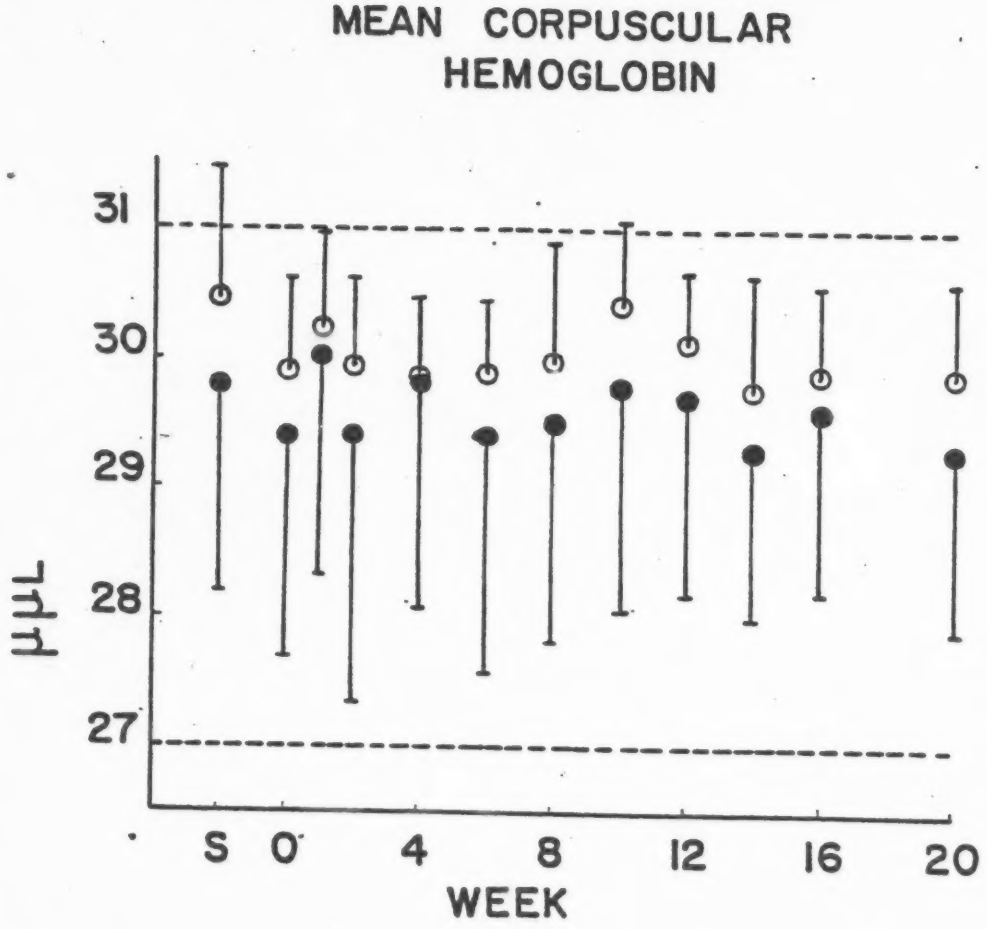


FIGURE 8a. ALBUMIN/ GLOBULIN RATIO
Normal range ----
Control group ●
Chlorite group ○

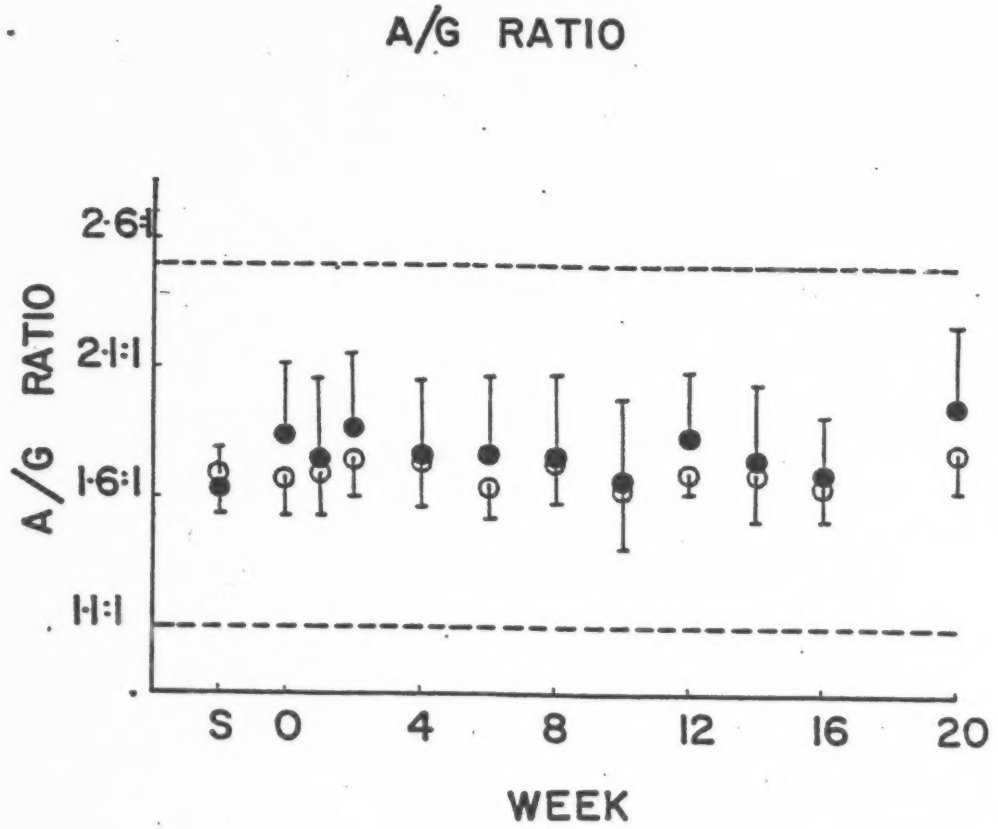


FIGURE 8b. ALBUMIN/ GLOBULIN RATIO
Normal range -----
Control group ●
Chlorate group ○

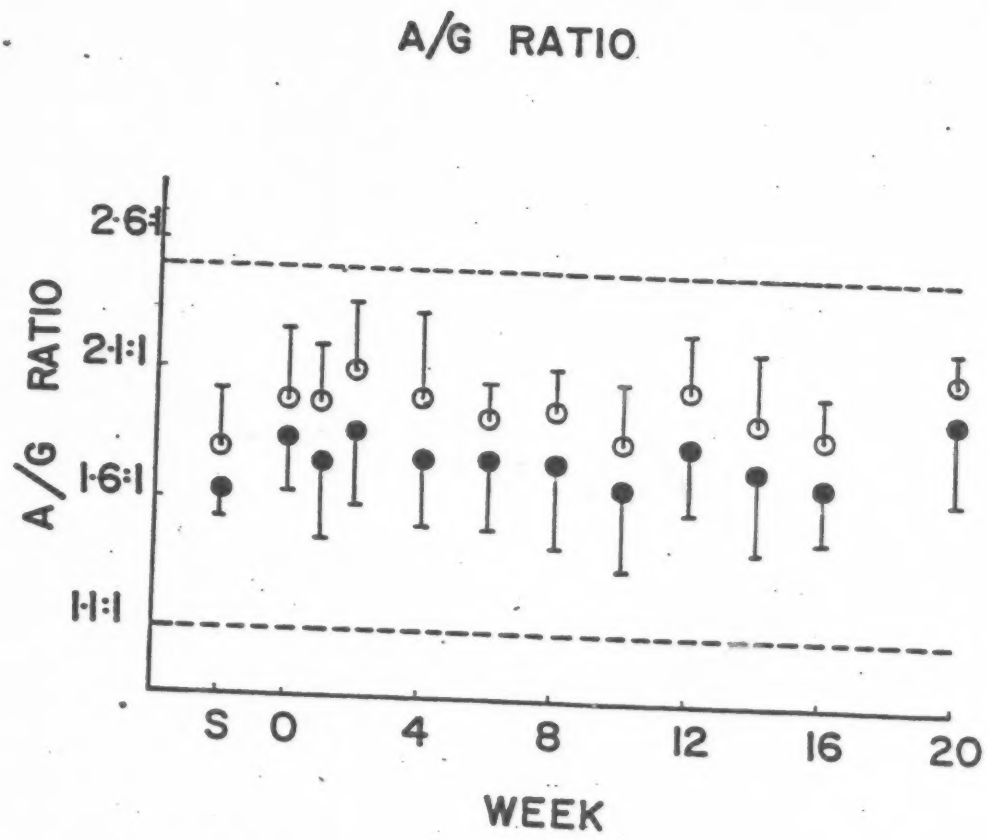


FIGURE 8c. ALBUMIN/ GLOBULIN RATIO
Normal range ----
Control group ●
Chlorine group ○

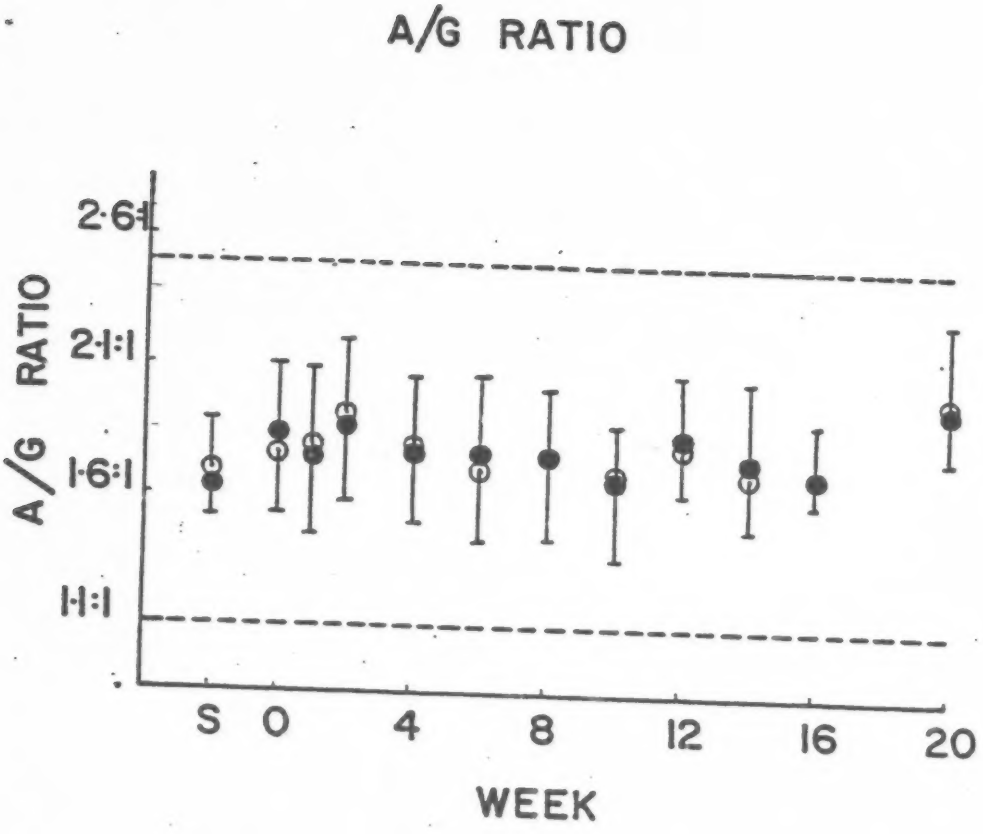


FIGURE 8d. ALBUMIN/
GLOBULIN RATIO
Normal range ----
Control group ●
Chlorine dioxide group ○

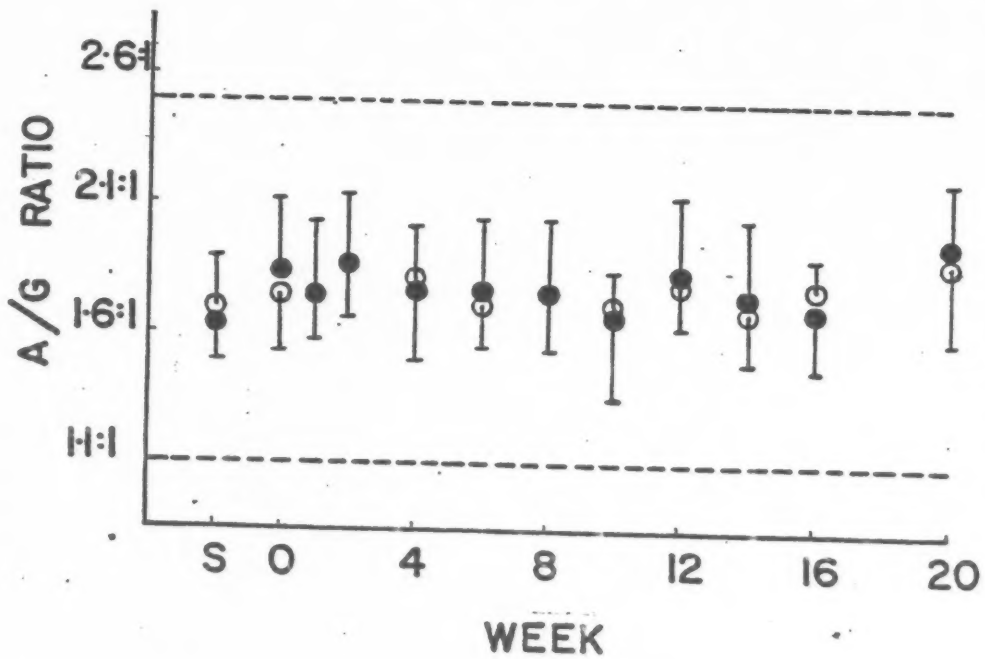


FIGURE 8e. ALBUMIN/ GLOBULIN RATIO
Normal range ----
Control group ●
Chloramine group ○

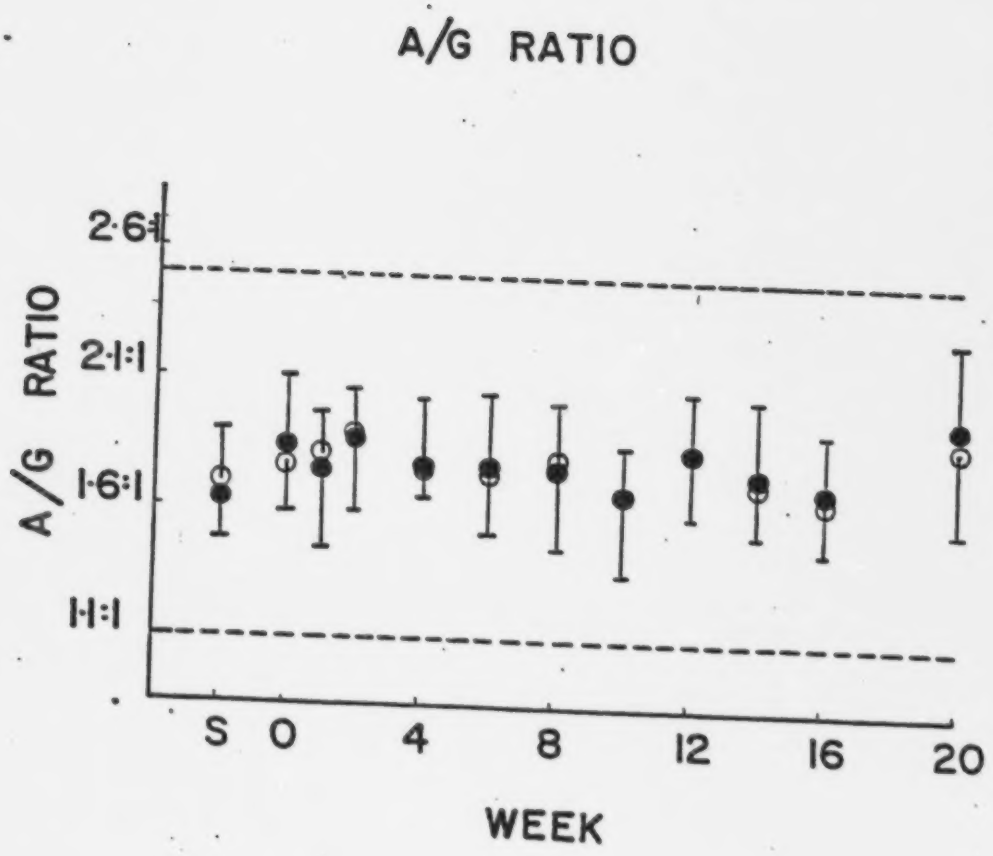


FIGURE 9a. METHEMOGLOBIN

Normal range ----
Control group ●
Chlorite group ○

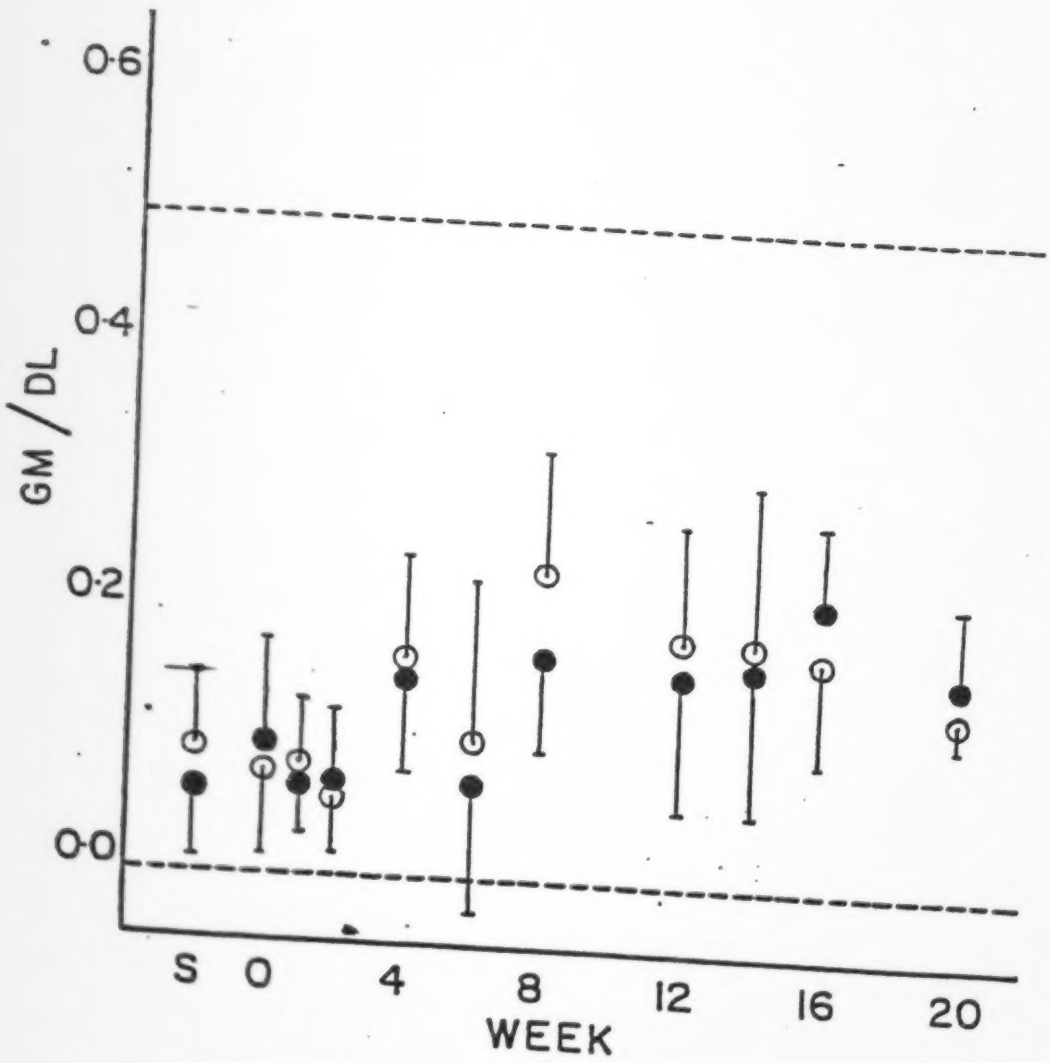


FIGURE 9b. METHEMOGLOBIN

Normal range ----
Control group ●
Chlorate group ○

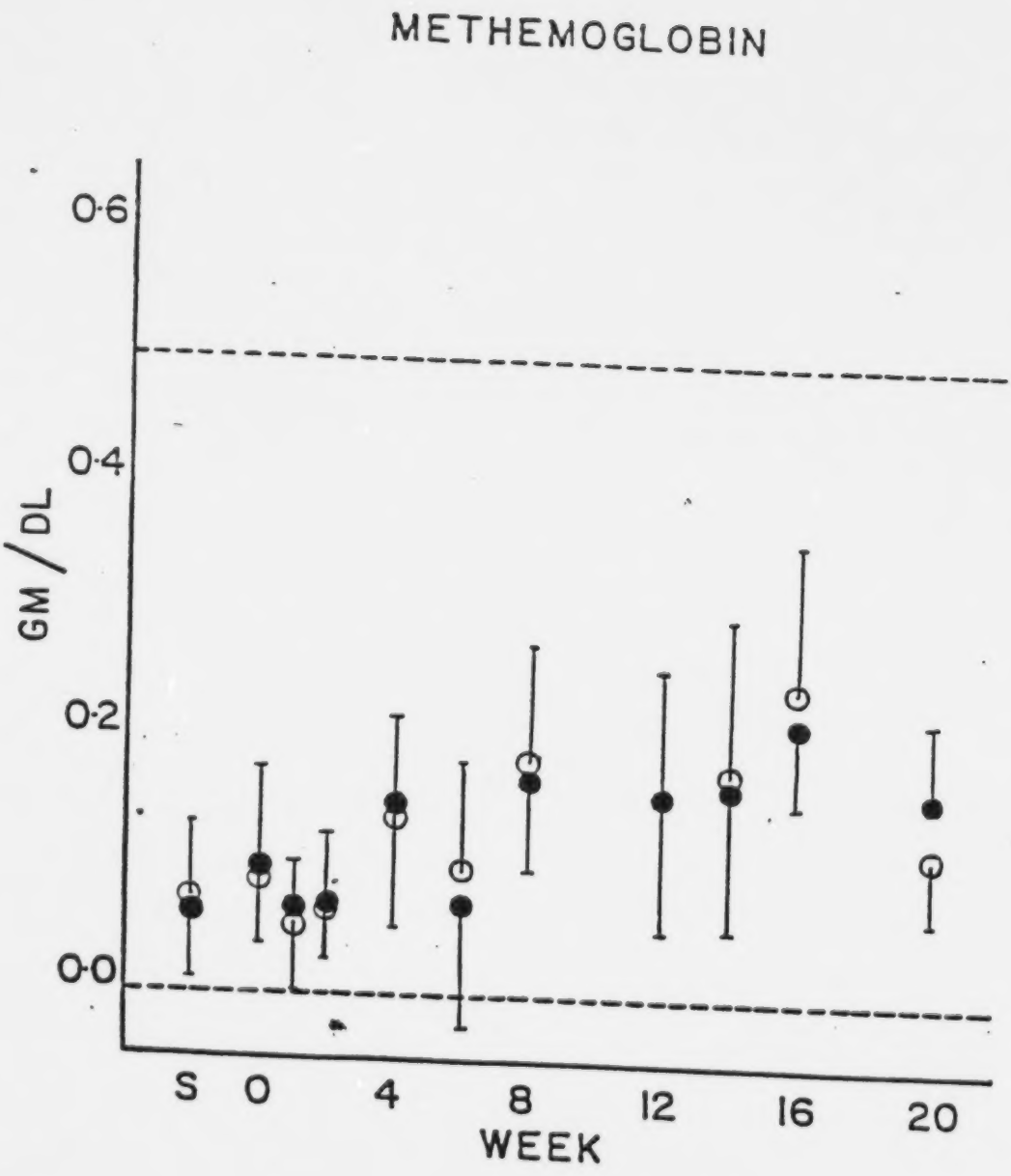


FIGURE 9c. METHEMOGLOBIN

Normal range ----
Control group ●
Chlorine group ○

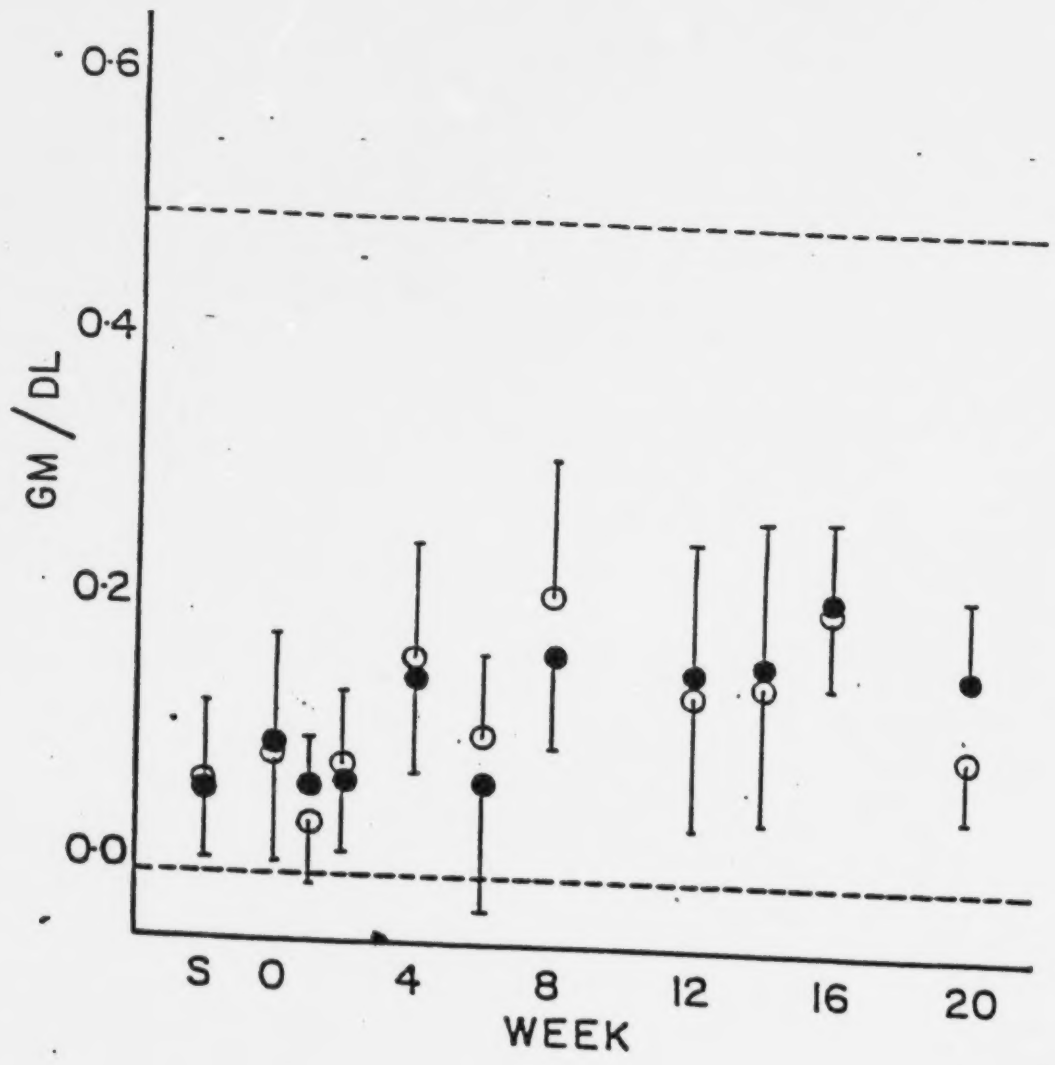


FIGURE 9d. METHEMOGLOBIN

Normal range ----
Control group ●
Chlorine dioxide group ○

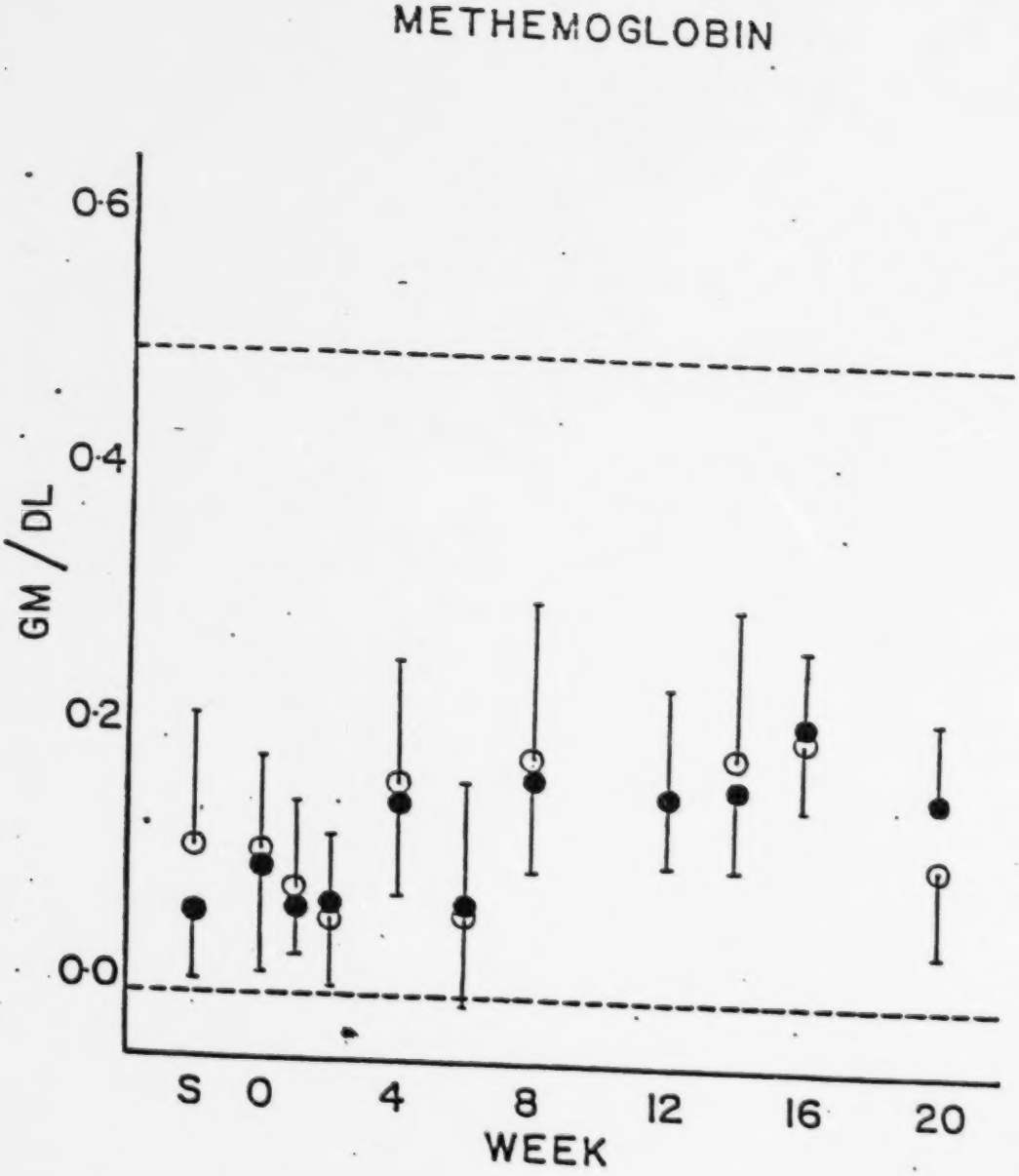


FIGURE 9e. METHEMOGLOBIN

Normal range ----
Control group ●
Chloramine group ○